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Carbohydrate-based peptidomimetics targeting neuropilin-1: synthesis, molecular docking study and *in vitro* biological activities

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ABSTRACT

Neuropilin-1 (NRP-1), a transmembrane glycoprotein acting as a co-receptor of VEGF-A, is expressed by cancer and angiogenic endothelial cells and is involved in the angiogenesis process. Taking advantage of functionalities and stereodiversities of sugar derivatives, the design and the synthesis of carbohydrate based peptidomimetics are here described. One of these compounds **(56)** demonstrated inhibition of VEGF-A_{165} binding to NRP-1 (IC_{50} = 39 μM) and specificity for NRP-1 over VEGF-R2. Biological evaluations were performed on human umbilical vein endothelial cells (HUVECs) through activation of downstream proteins (AKT and ERK phosphorylation), viability/proliferation assays and *in vitro* measurements of anti-angiogenic abilities.

1. Introduction

Angiogenesis or formation of new blood vessels requires the binding of signaling molecules, such as vascular endothelial growth factor (VEGF), which is one of the most specific and important growth factors involved in this process.[1,2] VEGF-A_{165} mediates its biological effects through receptors located on the endothelial cells, *i.e.* VEGFR-1 (Flt-1) and VEGFR-2 (Flk-1/KDR). VEGF-A_{165} is overexpressed by a wide variety of human tumors and this overexpression has been correlated with invasion and metastasis.[3]

Interestingly, Neuropilin-1 (NRP-1), a receptor protein firstly described in neuronal guidance,[4] is involved in a wide range of physiological and pathological processes including angiogenesis.[5] This transmembrane protein was found to be a co-receptor of VEGF-A_{165} acting together with VEGFR-2 via its NRP-1 b1/b2 domain, resulting in increased affinity of VEGF-A_{165} for the extracellular domain of VEGFR-2.[6] VEGF-A_{165} can also contribute to VEGFR-2/NRP-1 complex formation via its binding through two different sites for both NRP-1 and VEGFR-2.[7] Moreover, independently of VEGFR-2, NRP-1 alone might transduce functional signaling as a result of VEGF binding.[8] NRP-1 is expressed on endothelial cells promoting tumor angiogenesis[6,9] and on tumor cells and has thus been identified as a potential target for anti-angiogenic therapies.[10,11] This receptor could also be a mediator of choice for cancer imaging. Few attempts to use NRP-1 in cancer imaging via a [99mTc]-labelled peptide,[12] multifunctional nanoparticles engineered with gadolinium chelates as MRI contrast agents,[13] and fluorescein-labeled peptides are reported in the literature.[14] More recently, NRP-1 targeting peptides were conjugated onto the surface of lipid microbubbles for molecular imaging of tumor angiogenesis.[15]

Targeting NRP-1 with small molecules mimicking VEGF-A_{165} is not very advanced, because of difficulty to mimic protein-protein interactions. Nevertheless, several peptides have been reported to modulate VEGF-A_{165}/NRP-1 binding. The first crystal structure of the b1 domain of the human NRP-1 was determined by Lee et al.[16] The role of the loops at b1 domain of human NRP-

1 as a target binding site for ligand interaction has also been highlighted by Vander Kooi *et al.* with their study of NRP-1 binding with tuftsin (TKPR), which is very similar to the VEGF-A_{165} *C*-terminus (DKPRR).[17] The *C*-terminal arginine of tuftsin contributes to the majority of interactions with NRP-1 and this was confirmed by a molecular modeling approach developed by Haspel *et al.*[18] The interaction of NRP-1-b1 domain with VEGF-A_{165} was recently elucidated by Parker *et al.*[19] Jia *et al.* have discovered a specific bicyclic peptide EG3287 antagonist of VEGF-A_{165} binding to NRP-1.[20] Heptapeptide ATWLPPR (A7R), selected by screening a phage display library was described as an effective antagonist and can be considered as a potent inhibitor of tumor angiogenesis.[21] NRP-1 targeting photodynamic therapy (PDT) using A7R as NRP-1 ligand associated with a porphyrin-like sensitizer has since been developed.[22] New peptides structurally related to VEGF-A_{165} exon-7 and -8 domains were recently designed and synthesized.[23]

However, peptidic compounds could suffer from poor bioavailability and instability[24] and the design of new organic molecules such as peptidomimetic derivatives seems an attractive alternative. In connection with our ongoing program on the design of sugar-based peptidomimetics, we described a few years ago the design, synthesis and *in vitro* biological evaluation of sugar-based peptidomimetics targeting NRP-1. Interesting compounds were obtained and one hit compound was identified (compound **1**, Chart 1a).[25] Simultaneously, a paper by Jarvis *et al.* described compound EG00229 as small molecule inhibitor of the NRP-1/VEGF-A_{165} binding and this molecule has recently shown *in vivo* activity towards cancer models (Chart 1a).[26] More recently, a fully non-peptidic compound comprising phenylbenzimidazole and benzodioxane moieties linked by a stable carboxythiourea spacer was identified.[27] New antagonists structurally-related to this compound were synthesized and evaluated toward VEGF-A_{165}/NRP-1 binding modulation.[28] Several drug-like compounds containing a common chlorobenzyloxy alkyloxy halogenobenzyl amine scaffold were identified by virtual screening for their efficient binding to NRP-1.[29]

The objective of the present work was the molecular design and synthesis of new NRP-1 ligands inspired by compound **1**.[25] The use of carbohydrates as scaffolds to construct bioactive compounds and peptidomimetics is a well-accepted concept since the pioneering works related to peptidomimetics of somatostatin.[30,31,32] We wanted to take advantage of functionalities and stereodiversities of sugar derivatives to develop new analogs of carbohydrate-based peptidomimetics. Thus, sugar scaffolds functionalizable at both ends and offering different spatial orientations for residues were investigated according to molecular modeling and the *in vitro* biological properties of the new ligands were evaluated.

2. Results and discussion

Sugar scaffolds A and B suitable for functionalization at both ends could arrange crucial residues in opposite spatial directions and were both investigated in this regard (Chart 1b). Firstly, compound **1** was modified stepwise, the importance of the two guanidine functions was studied and the effect of hydrocarbon linker lengths separating the guanidinium groups was explored (first series). A second series with an arginine anchored at C1 was developed according to docking study. In the meantime, synthetic compounds were used to get crystals of NRP-1/ligand complex. The binding affinity of new derivatives was performed by ELISA and the effects of the most potent ligands were evaluated on HUVECs through activation of downstream proteins, viability assays and *in vitro* angiogenic abilities.

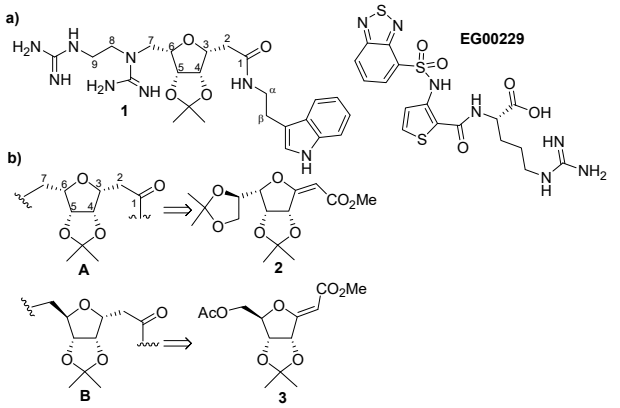


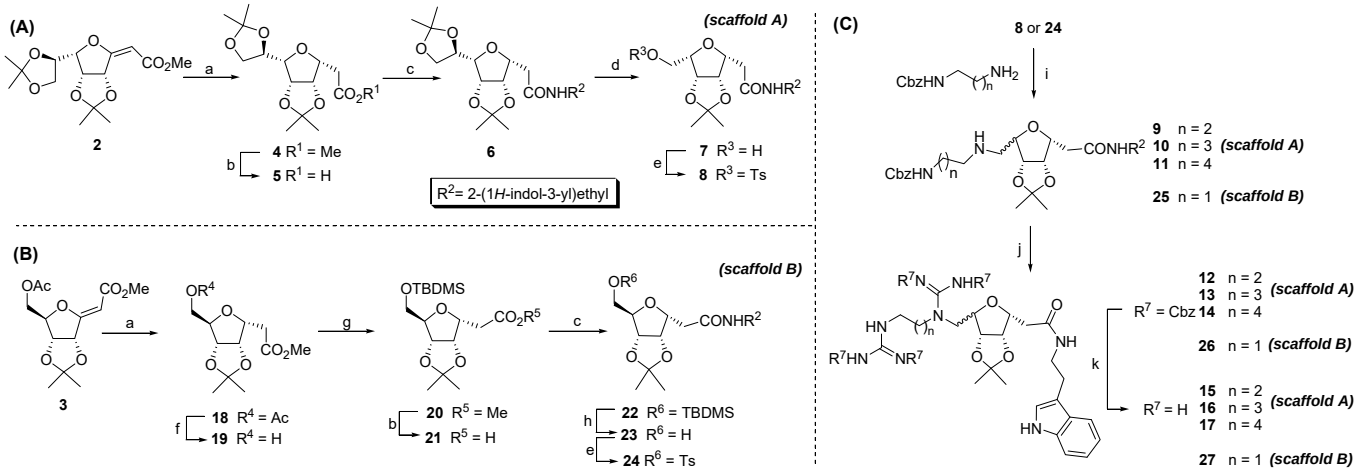
Chart 1. a) Structure of compound **1** and EG00229. b) Structure of scaffolds A and B functionalizable at both ends and structure of starting *exo*-glycals **2** and **3**.

2.1. Synthetic Chemistry

The *C*-glycosidic scaffold A which was used for compound **1** synthesis was obtained from the known *exo*-glycal **2** available in excellent yield *via* a Wittig reaction on the D-gulono-1,4-lactone.[33] In the same way, scaffold B was obtained from *exo*-glycal **3** directly available from the corresponding D-ribono-1,4-lactone. The first series aimed at synthetizing new peptidomimetic compounds close to compound **1** structure. The impact of the spatial disposition of both guanidium groups and 2-(indolyl-3-yl)ethyl residue was evaluated by synthesizing a compound based on the sugar scaffold B. Moreover, while maintaining compound **1** central core (scaffold A) with the 2-(indol-3-yl)ethyl residue at C1, the effect of the linker length between both guanidinium groups was considered. The replacement of one of the guanidium functions by a triazole moiety acting as a stable linker[34-36] was envisioned.

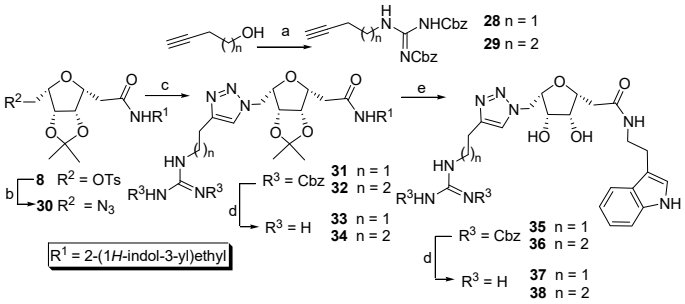
The synthetic pathway for the first series required the preparation of the key tosylates **8** and **24** (Scheme 1A and 1B). The known *exo*-glycal **2** was converted to *C*-glycoside **4** by stereoselective reduction of the double bond.[37] Subsequent saponification of **4** followed by coupling with tryptamine led to amide **6**. Alcohol **7** was prepared by a multistep sequence including the selective removal of the exocyclic isopropylidene acetal under acidic conditions, $NaIO_4$ mediated oxidative cleavage and reduction of the resulting aldehyde. The alcohol **7** was then converted in the corresponding tosylate **8** in excellent yield. In the same way, *exo*-glycal **3** was converted in *C*-glycoside **18**. After changing acetate protecting group at O7 for a *tert*-butyldimethylsilyl ether, subsequent saponification of **20** led to the key acid **21** in good yield. Amide **22** was prepared by coupling with tryptamine. Removal of the *tert*-butyldimethylsilyl group was achieved by TBAF and the resulting alcohol was converted in tosylate **24** in 87% yield.

Refluxing tosylates **8** and **24** with different commercially available monoprotected diaminoalkanes (n = 2, 3 and 4 for **8** and n = 1 for **24**, Scheme 1C) in a sealed flask led to intermediates **9**, **10**, **11** and **25**. Free amines obtained in quantitative yields after removal of benzyloxycarbonyl group were treated with *N,N'*-bis(benzyloxycarbonyl)-*S*-methylisothiourea[38] in DMF. Bis-guanidinylated derivatives **12**, **13**, **14** and **26** were obtained in modest yields, resulting from the reaction of both primary and secondary amines. Finally, removal of protecting groups afforded the expected bis-guanidinoglycosides **15**, **16**, **17** and **27**. Attempts to remove the 4,5-isopropylidene group under different acidic conditions were unsuccessful, the number of nitrogen atoms obviously preventing oxygen protonation required for acetal deprotection.



Scheme 1. Synthetic route to compounds **8 (A)**, **24 (B)** and **15-17** and **27 (C)**. Reagents and conditions: (a) H_2, 15 psi, Pd/C, EtOAc, 25°C, 24h, **4** 98%, **18** 85 %; (b) LiOH, THF/H_2O (3/1), 25°C, 18h, **5** 98%, **21** 65%; (c) tryptamine, EDC, CH_2Cl_2, 25°C, 18h, **6** 80%, **22** 80%; (d) i: 1N HCl, MeOH, 0°C to 25°C, 8h; ii: $NaIO_4$, MeOH, 25°C, 18h, iii: $NaBH_4$, MeOH, 0°C, 1h, 72% for three steps; (e) TsCl, Et_3N, DMAP, CH_2Cl_2, 25°C, 24h, **8** 90%, **24** 87%; (f) Na^0, MeOH, 0°C, 1h, 97%; (g) TBDMSCl, imidazole, DMF, 25°C, 24h, quantitative yield; (h) TBAF, THF, 0°C, 18h, 95%; (i) *i*PrOH, 100 °C, sealed tube, 48h, **9** 72%, **10** 70%, **11** 80%, **25** 82%; (j) i: H_2, 30 psi, Pd/C, MeOH, 18h, quantitative yields, ii: *N,N'*-bis(benzyloxycarbonyl)-*S*-methylisothiourea, $HgCl_2$, Et_3N, DMF, 25°C, 14h, **12** 25%, **13** 25%, **14** 35%, **26** 30%; (k) H_2, 30 psi, Pd/C, MeOH, 18h, **15** 90%, **16** 85%, **17** 94%, **27** 90%.

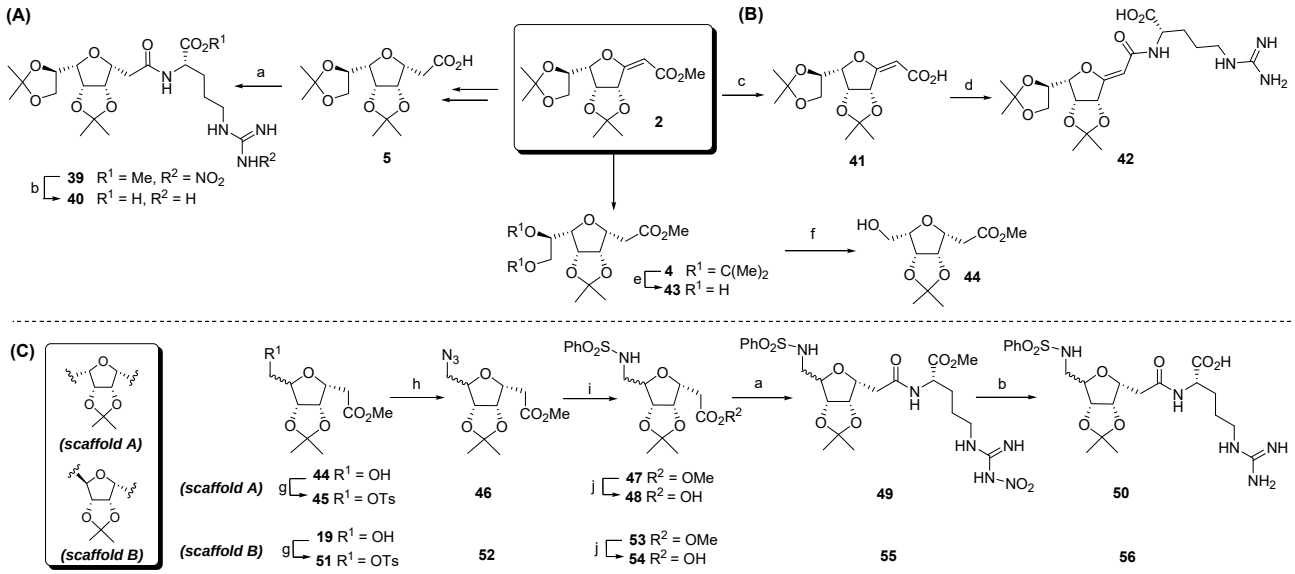
On the basis of structure **1**, we next focused our attention on the replacement of the guanidine function anchored on the secondary amine by a 1,2,3-triazole moiety bearing guanidylated arms (Scheme 2). This synthetic approach required the preparation of two guanidylated alkynes **28** and **29** obtained by a Mitsunobu reaction. The *C*-glycoside **30** bearing an azido group on C7 was obtained by reaction of sodium azide with tosylate **8**. The synthesis of the 1,4-substituted-1,2,3-triazole moiety was next envisioned between azide **30** and alkynes **28** and **29** in a 1/1 H_2O/CH_2Cl_2 mixture, which was the more appropriate solvent in this case.[39,40] Compounds **31** and **32** were obtained in good yields. Removal of benzyloxycarbonyl protecting groups was achieved by hydrogenolysis and led to **33** and **34** in nearly quantitative yields. Removal of 4,5-isopropylidene protecting group by aqueous trifluoroacetic acid was performed and fully deprotected derivatives **37** and **38** were then obtained by hydrogenolysis.



Scheme 2. Synthetic route to compounds **33-34** and **37-38.** Reagents and conditions: (a) DEAD, PPh_3, *N,N'*-bis(benzyloxycarbonyl)guanidine, toluene, 0°C then 25°C, 4h, **28** 92%, **29** 89%; (b) NaN_3, DMF, 80°C, 2 days, 88%; (c) $CuSO_4$, sodium ascorbate, **28** or **29**, CH_2Cl_2, H_2O, 25°C, 24h, **31** 80%, **32** 70%; (d) H_2, 30 psi, Pd/C, MeOH, 18h, **33** 90%, **34** 90%, **37** 80%, **38** 95%; (e) TFA/H_2O (1/1), 25°C, 10h, **35** 85%, **36** 53%.

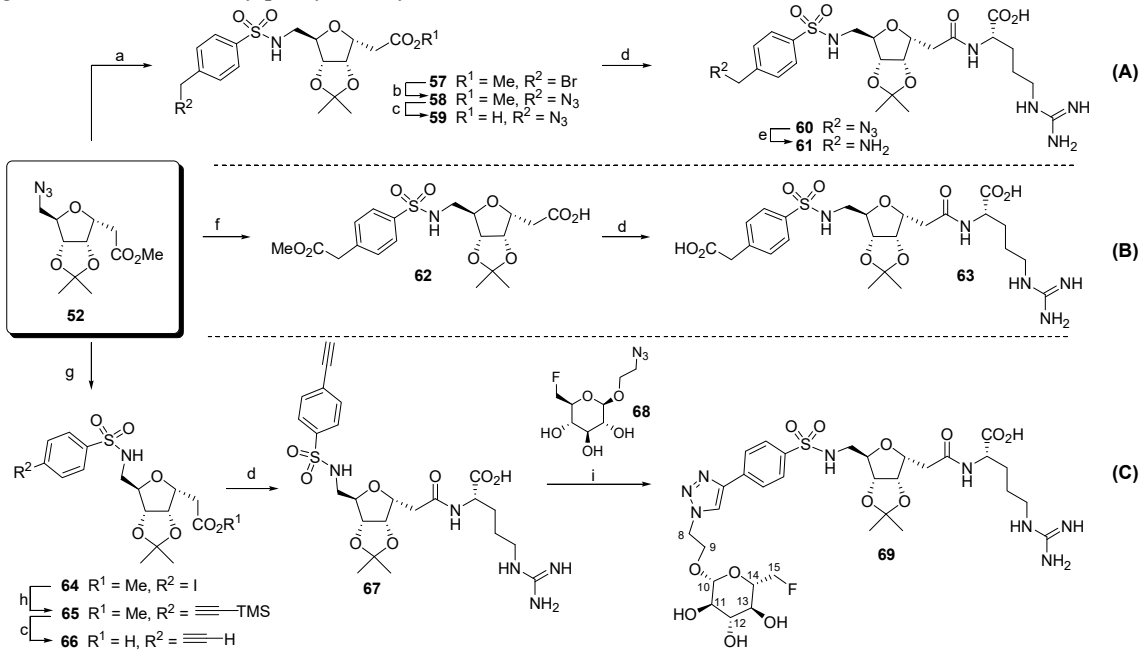
The synthetic plan for the second series implied the introduction of an L-arginine at C1 and the anchoring of a hydrophobic residue at C7. The coupling of *C*-glycosyl compound **5** with N^{ω}-nitro-L-arginine methyl ester led to amide **39** (Scheme 3A). The carboxylic acid and guanidinium function of arginine were successively deprotected by classical methods. We next investigated the impact of a more rigid system based on *C*-glycosylidene derivative obtained from *exo*-glycal **2** (Scheme 3B). To this end, using a method for methyl ester deprotection which did not affect the anomeric double bond was mandatory. This was achieved by using lithium iodide in pyridine. The resulting vinylic acid **41** was then coupled with L-arginine methyl ester without protection on its lateral chain. An isomerization of the double bond was observed and two isomers E/Z were obtained in an approximatively 1/1 ratio. This isomerization could obviously be attributed to reversible addition of HOBt on the anomeric carbon of activated *exo*-glycal, prone to 1,4-additions.[33] At this point, the purification of the isomeric mixture was not possible by normal or reverse phase chromatography. This mixture was thus subsequently treated by lithium iodide in pyridine and reverse phase purification led to isolation of **42**, which is the only one obtained as pure material.

Guided by published works[25-28] highlighting the favorable impact of an hydrophobic group and docking studies pointing out the benefit of a supplementary chemical groups such as a phenyl (see 2.2), an aromatic residue was linked to C7 via a sulfonamide linkage. This required the preparation of the key intermediate tosylates **45** and **51** obtained from **44** and **19** (Scheme 3C). The selective removal of the exocyclic isopropylidene acetal of **4** under acidic conditions led to diol **43** subsequently transformed in alcohol **44**. Substitution with sodium azide carried out in DMF led to azido derivatives **46** and **52** in 84 and 97% yields respectively. Catalytic hydrogenation afforded primary amines which were treated with benzene sulfonyl chloride in pyridine to give phenylsulfonamides **47** and **53** in good yields. We next managed the introduction of L-arginine at C1 and compounds **49** and **55** were obtained in 84% and 71% yields respectively. Finally, fully deprotected derivatives **50** and **56** were obtained in good yields after methyl ester and arginine side chain protection removal.



Scheme 3. Synthetic route to compounds **40 (A), 42 (B)** and **50** and **56 (C).** Reagents and conditions: (a) H-L-Arg(NO_2)-OMe.2HCl, HATU, DIEA, DMF, 0°C then 25°C, 24h, **39** 50%, **49** 84%, **55** 71%; (b) i: LiOH, THF/H_2O 3/1, 25°C, 18h, ii: H_2, 40 psi, Pd/C, MeOH, 18h, **40** 70%, **50** 82%, **56** 70% for two steps; (c) LiI, pyridine, 120°C, 24h, 50%; (d) i: L-Arg-OMe.HCl, HATU, DIEA, DMF, 0°C then 25°C, 24h; ii: LiI, pyridine, 120°C, 24h, 33%; (e) 1N HCl, MeOH, 0°C then 25°C, 6h, 92%; (f) i: $NaIO_4$, MeOH, 25°C, 18h, ii: $NaBH_4$, MeOH, 0°C, 1h, 93% for two steps; (g) TsCl, Et_3N, DMAP, CH_2Cl_2, 25°C, 18h, **45** 92%, **51** 98%; (h) NaN_3, DMF, 80°C, **46** 84%, **52** 97 %; (i) i: H_2, 15 psi, Pd/C, MeOH, 8h, quantitative yields, ii: $PhSO_2Cl$, pyridine, 25°C, 18h, **47** 80%, **53** 83%; (j) LiOH, 3/1 THF/H_2O, 25°C, 18h, **48** 90%, **54** 96%.

Molecular modeling studies highlighted that presence of a negative or positive charge in *para* position on the aromatic ring could led to improved affinity (see 2.2). Taking into account preliminary inhibition results (see 2.3), we thus planned to functionalize **56** with substituents able to make additional hydrogen bonds or salt bridges in the receptor binding site. In this regard, an aminomethyl and a hydroxycarboxymethyl groups were selected (Scheme 4A and 4B). Starting from azido derivative **52**, the coupling of the 4-bromomethylphenylsulfonyl chloride in dichloromethane led to the bromomethyl derivative **57**. Compound **58** was then obtained in quantitative yield by nucleophilic substitution of the bromine with sodium azide in DMF. Coupling with L-arginine methyl ester and treatment with lithium hydroxide gave acid **60** in 86% yield for two steps. Finally, fully deprotected derivative **61** was obtained by catalytic hydrogenation of azido group.



Schemes 4. Synthetic route to compounds **61 (A)**, **63 (B)** and **69 (C)**. Reagents and conditions: (a) i: H_2, 15 psi, Pd/C, MeOH, 8h, quantitative yield , ii: 4 bromomethylphenylesulfonyl chloride, CH_2Cl_2, Et_3N, 25°C, 0.5h, 61%; (b) NaN_3, DMF, 60°C, 15h, quantitative yield; (c) LiOH, THF/H_2O, 25°C, 8h, **59** quantitative yield, **66** 82%; (d) i: L-Arg-OMe.2HCl, HATU, DIEA, DMF, 0°C then 25°C, 18h, ii: LiOH, THF/H_2O, 25°C, 8h, **60** 86% for two steps, **63** 54% for two steps, **67** 67% for two steps; (e) H_2, 15 psi, Pd/C, MeOH, 18h, 87%; (f) i: LiOH, THF/H_2O, 25°C, 6h, ii: H_2, 15 psi, Pd/C, MeOH, 8h, iii: methyl 2-(4-chlorosulfonylphenyl)acetate, pyridine, 25°C, 2h, 30% for three steps; (g) i: H_2, 15 psi, Pd/C, MeOH, 8h, quantitative yield, ii: 4-iodophenylesulfonyle chloride, pyridine, 25°C, 6h, 82%; (h) ethynyltrimethylsilane, CuI, $PdCl_2(PPh_3)_2$, Et_3N, 80°C, 2h, 80%; (i) **68**, $Cu(OAc)_2$, sodium ascorbate, *t*BuOH/H_2O, 25°C, 18h, 77%.

The synthesis of compound **63** required a slightly modified pathway, notably the saponification of methyl ester **52** before introduction of the sulfonyl chloride. After saponification with lithium hydroxide and catalytic hydrogenolysis of azido ester **52** with Pd/C, sulfonamide **62** was obtained by reaction with methyl 2-(4-chlorosulfonylphenyl)acetate. Coupling with L-arginine methyl ester and removal of both methyl ester functions led to the diacid **63**. In connection with ongoing work in our group[41] concerning conjugation of biomolecules with sugar derivatives, compound **56** was coupled with the 2-azidoethyl-6-fluoro-β-D-glucopyranoside **68**. Indeed, 2-azidoethyl-6-fluroglycosides are easily prepared[41b] and are valuable prosthetic groups for fast and easy labelling of peptides or peptidomimetics by copper(I)-catalyzed azide–alkyne cycloaddition.[36] To this end, derivative **64** was obtained by coupling 4-iodobenzenesulfonyle chloride (Scheme 4C). Sonogashira reaction was then performed with trimethylsilylacetylene. Subsequent removal of methyl ester and trimetysilyl protecting groups was performed and led to alkyne **66** in good yield. As described above, L-arginine methyl ester was introduced on C1 position. The copper(I)-catalyzed azide–alkyne cycloaddition was performed between alkyne **67** and azide **68** and led to fluoro derivative **69** in 77% yield.

2.2. Crystallographic study and molecular modeling

Cocrystallization screenings of NRP-1-b1 fragment were carried out with compound **1** and four others compounds, namely compounds **27**, **40**, **50** and **56** (Chart 2).

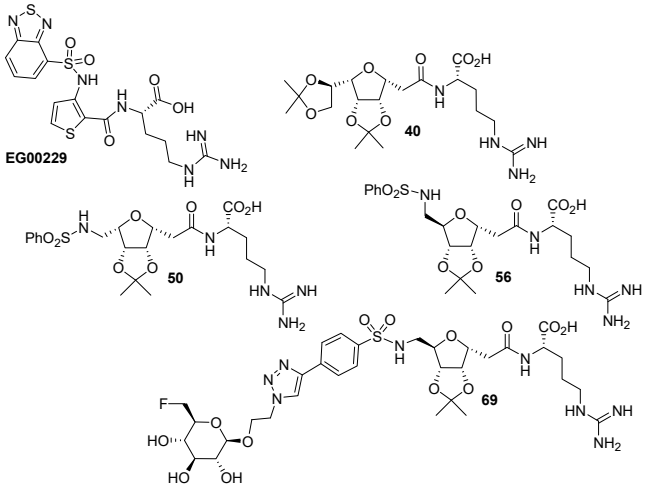


Chart 2. Structures of EG00229 and compounds **40**, **50**, **56** and **69**.

Crystals were obtained in presence of compound **56**. They exhibited the same tetragonal crystal packing as those of the free protein fragment[16] but the resolution of the data was substantially improved (1.45 vs. 1.90 Å, see SI Table S1). The structure revealed that the crystallized protein did not form a complex with the synthesized compound. Instead, a bicine molecule was found in the VEGF-A_{165} binding cleft (Figure 1a). The bicine sodium salt was used as pH buffer in the crystallization condition. A sodium atom in octahedral geometry was also found, it is interacting with a bicine hydroxyl-group and five water molecules. The ternary amine and carboxylic group of bicine are hydrogen-bonded to the Tyr353 and Thr349 side-chains, respectively. The bicine molecule also formed interactions with residues of a symmetry related protein molecule (see SI Figures S1, S2). Several methylene hydrogen atoms of bicine form hydrophobic H...Pi and H...H interactions with Tyr297 and Trp301. The Asp320 residue, which forms an important salt bridge with an arginine in the NRP-1/VEGF-A_{165} biological complex[19] and in the complex with EG00229[26a] was in the present structure in interaction with the Na^+ ion through a water bridge. This NRP-1-b1/bicine complex highlighted the conservation of the anchoring residues of the VEGF-A_{165} binding cleft.

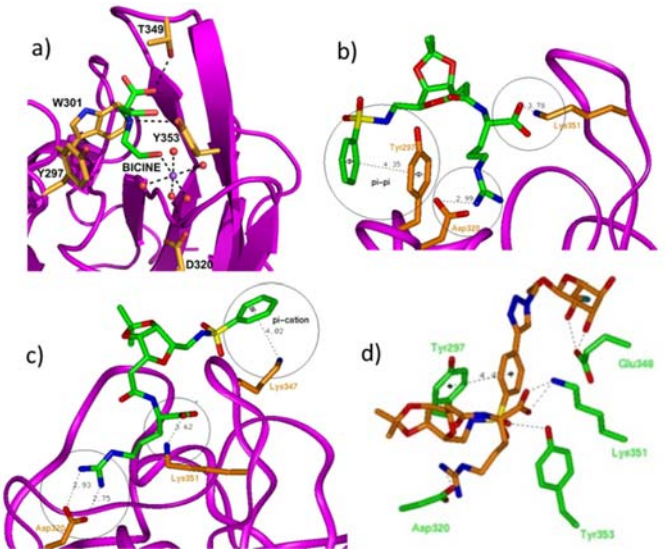


Figure 1. (**a**) Crystal structure of bicine/NRP-1-b1 complex. (**b**) Docking of compound **56** showing the pi-pi supplementary interaction. (**c**) Docking of compound **56** showing the pi-cation supplementary interaction. (**d**) Docking of compound **69**.

The crystal structures of NRP-1-b1/ligand complexes provided us additional informations to guide an *in silico* optimization of our synthesized ligands. We undertook optimization of structures by molecular modeling and compound **40** was chosen as a starting point. It appeared from preliminary docking calculations that all the obtained poses of the first docked molecule **40** were anchored within the binding site in the same way as the PDB 3I97 EG00229 ligand (Chart 2), namely through a strong ionic interaction involving the guanidinium moiety of the ligand and the protein Asp320. It should be noted that the best pose obtained for the EG00229 compound itself was exactly similar to the pose found in the X-ray structure, therefore validating our docking procedure.[26a] Depending on poses, several additional favorable H-bond interactions were detected with residues containing hydroxyl groups such as Tyr353, Tyr297, Thr349 and Ser346. It is also noteworthy that these interacting residues are similar to those found in the bicine/NRP1-b1 structure. The best docking poses of **40** and EG00229 were next refined using short 10 ns molecular dynamics runs. The analysis of the different trajectories confirmed the robustness of the ligand guanidine…Asp320 salt bridge which was observed as stable during all simulations (Figure 2a). A supplementary ionic interaction was also established after several ns of the MD simulations stabilizing the position of the arginine terminal-carboxyl of the ligands with the ammonium group of Lys351 (Figure 2b).

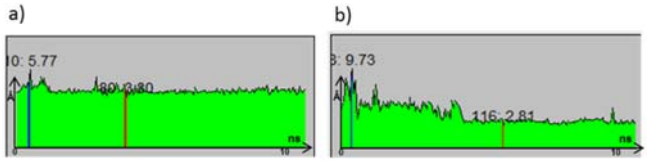


Figure 2. (**a**) Molecular dynamics of the protein/**40** complex runs confirming the robustness of guanidine…Asp320 side chain salt bridge; (**b**) Molecular dynamics runs confirming the robustness of arginine terminal-carboxyl …Lys351 side chain salt bridge; vertical axis: interatomic distances in Å.

Such an interaction between an exposed Lys side chain with the ligand, while in competition with the solvent, is nevertheless observed in many protein/ligand complexes such as the kinases.[42] In the case of the X-ray structure complex with EG00229, the presence of an intramolecular hydrogen bond involving the arginine terminal-carboxyl of EG00229 precluded an interaction with Lys351 in the complex. Comparing the behavior of **40** and EG00229 during these MD simulations showed that **40** could present additional stabilizing interactions on the condition to add supplementary chemical groups such as a phenyl moiety at the C7 position. Furthermore, both C6 stereoisomers were considered (scaffolds A and B, Chart 1). Consequently, we checked this hypothesis with both new synthesized compounds **50** (scaffold A) and **56** (scaffold B) which were submitted to the same docking and short MDs protocols. Comparing the interactions found during these MD simulations between **40**, **50** and **56** clearly showed that compound **56** should be a better compound, this behavior being mostly due to π-π and π-cation additional interactions coming from the added phenyl ring with Tyr297 or Lys347 side chains (Figure 1b and 1c). These interactions are observed during most of the simulation. According to these simulations, it can also be suggested that expanding the phenyl group with positive or negative charged groups (compounds **61** and **63**) or with longer chains such as the one found in **69** could give the possibility to attract the Lys347 or Glu348 or Glu312 charged side chains (Figure 1d).

2.3. Biological evaluation

The binding of new derivatives to recombinant NRP-1 protein was determined using a competition assay initially described by Tirand *et al.*[43] Binding of compounds to NRP-1 was assessed using biotinylated VEGF-A_{165} (bt-VEGF-A_{165}), in competition, or not, with an excess of compounds or non labelled VEGF-A_{165}. Tuftsin was used as positive control (IC_{50} = 25 µM). A preliminary screening with 100 µM inhibitor concentrations was performed. For the most active compounds (inhibition >40% at 100 µM, Table 1), the IC_{50} value was determined with concentrations ranging from 10 µM to 500 µM. VEGF might crosslink VEGF-R2 and NRP-1[26] and it was recently demonstrated that NRP-1 could interact directly with VEGF-R2 without VEGF.[6b] Thus, it was appropriate to compare binding of new derivatives with NRP-1 and VEGF-R2. The binding of the four best compounds to VEGF-R2 (KDR) were evaluated using a similar competition assay, showing the selectivity for NRP-1 (Table 2).

Table 1. Binding inhibition of bt-VEGF-A_{165}/NRP-1 (%) in the presence of new compounds for a fixed concentration of 100 µM and IC_{50} values.

Entry	Compound	Binding inhibition of bt-VEGF-A_{165} at 100 µM (%)	IC_{50} (µM)[a]
1	VEGF-A_{165}	100± 1	-
2	**15**	5 ± 2	nd
3	**16**	39 ± 2	nd
4	**17**	52 ± 1	120
5	**27**	26 ± 3	nd
6	**33**	5 ± 3	nd
7	**34**	18 ± 2	nd
8	**37**	37 ± 1	nd
9	**38**	4 ± 7	nd
10	**40**	43 ± 2	187 ± 19
11	**42**	44 ± 1	181± 22
12	**50**	55 ± 2	88 ± 8
13	**56**	67 ± 1	39 ± 1
14	**61**	40 ± 2	134 ± 2
15	**63**	18 ± 1	nd
16	**69**	57 ± 1	69 ± 2

[a]: IC_{50} values were measured for all compounds showing inhibition >40% at 100 µM; results were obtained from three independent experiments, each performed using triplicate determinations at each concentration of compound and are presented as the mean +/- SEM; R^2 values ranged from 0.9739 to 0.9927; nd, not determined

Table 2. Binding inhibition of bt-VEGF-A_{165}/KDR (%) in the presence of compounds **50**, **56**, **61** and **69** for a fixed concentration of 100 µM and IC_{50} values.

Entry	Compound	Binding inhibition of bt-VEGF-A_{165} for 100 µM (%)	IC_{50} (µM)[a]
1	VEGF-A_{165}	96 ± 5	-
2	**50**	5 ± 6	> 500
3	**56**	15 ± 13	> 500
4	**61**	0 ± 13	> 500
5	**69**	26 ± 7	306 ± 5

[a]: IC_{50} values were measured for compounds **50**, **56**, **61** and **69**, concentrations ranging from 10 µM to 500 µM; results were obtained from three independent experiments, each performed using triplicate determinations at each concentration of compound and are presented as the mean +/- SEM; R^2 value for compound **69**: 0.9911.

In order to determine the biological activity of compounds on endothelial cells, human umbilical vein endothelial cells (HUVECs) expressing VEGF-R1, VEGF-R2 and NRP-1 were used. It was previously demonstrated that the inhibition of VEGF-A_{165} binding to NRP-1 had effect on VEGF-A_{165} ability to activate VEGFR-2 (KDR).[20] Compounds

50, **56**, **61** and **69**, which have the higher affinities for NRP-1 (Table 1) were selected for their biological evaluation on HUVECs. Binding of compounds to NRP-1 was evaluated by the activation of PI3K/AKT and MAPK downstream signaling pathways, through phosphorylation of AKT, a serine/threonine protein kinase and extracellular signal-regulated protein kinases 1 and 2 (ERK1/2), respectively. Both are involved in NRP-1/VEGFR-2 signaling pathways. The cellular binding of the different compounds to NRP-1 led to a mean of 40% decrease of ERK phosphorylation (ratio ranging from 0.61 to 0.68, Figure 3A et 3B) compared to tuftsin (ratio of 0.88), the natural ligand of NRP-1. In contrast, an increase of AKT phosphorylation was observed for each ligand as well as for tuftsin. It should be noted that the most relevant effect was obtained for compound **56** (ratio 2.52), whereas compound **50** showed a lesser effect (ratio 1.56). Effects on HUVECs viability were measured by metabolic assay (WST) (Figure 3C). Modifications observed for signaling pathways ERK1/2 and AKT did not lead to detrimental effect on the viability of endothelial cells whatever the tested concentration.

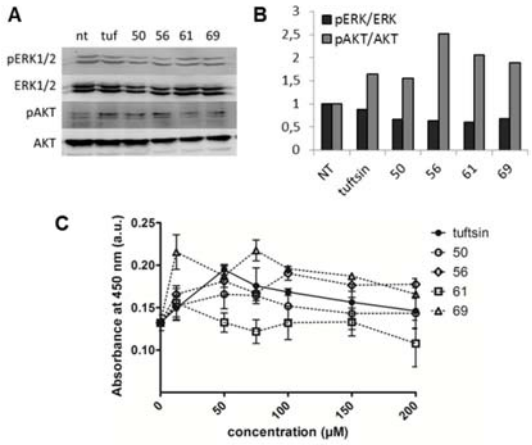


Figure 3. **A**: Effect of the different compounds (c = 50 µM) on the activation of downstream proteins of NRP-1 signaling pathways (pERK1/2 and pAKT) evaluated by western blotting. **B**: Ratio of phosphorylated protein reported to total protein. Optical densities of each band have been quantified, the expression ratio have been normalized with value of non-treated cells (nt) as reference. tuf = cells treated with tuftsin. **C**: Effect of the different compounds on HUVECs viability measured by a metabolic test (WST) at different concentrations from 12.5 µM to 200 µM. No statistically significant difference observed between the different compounds. *n =3*.

The anti-angiogenic properties were evaluated through the *in vitro* ability of HUVECs to form tubules-like capillaries on a basement matrix (Figure 4). *In vitro* angiogenesis assay using HUVEC seeded on Matrigel is a well-established assay to screen the angiogenic effect of many substances. Among the tested compounds, **69** demonstrated a wide effect on tubules formation. The effects of **69** could be compared to tuftsin and were relatively close to those observed with bevacizumab, a humanized anti-VEGF monoclonal antibody used nowadays in clinic for cancer therapy and used here as positive control. Inhibition of tubules-like capillaries was also observed to a lesser extent with compound **61**.

HUVECs ability to migrate through a basement membrane matrix was measured *via* an invasion assay and most of tested compounds showed an unexpected slight pro invasive effect (see SI, Figure S3).

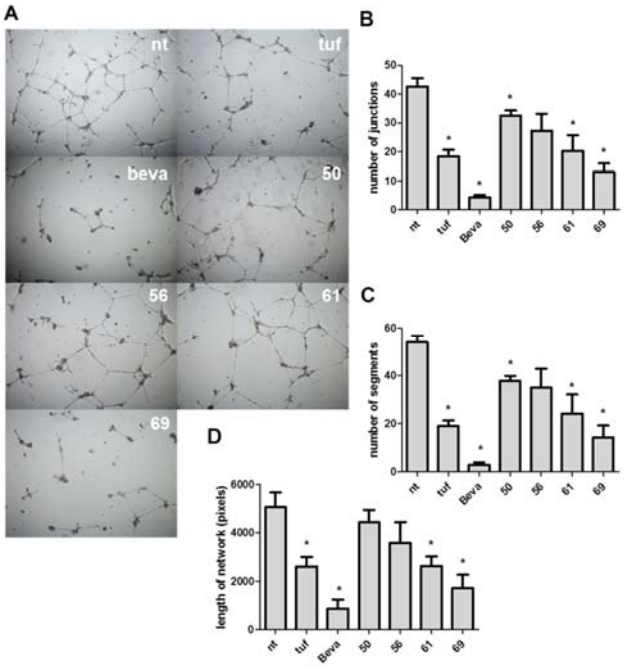


Figure 4: Effect of the different compounds (c = 50 µM) on HUVECs ability to form capillaries-like structures on basement membrane matrix. **A**: Images were representative of three independent experiments of endothelial cells cultured on Matrigel™ in presence of the different compounds. The junctions number. **B** segments number **C** as well as the total segments length **D** were quantified by Angiogenesis Analyser for ImageJ. nt, non treated; tuf, tuftsin; beva, bevacizumab. Results are presented as mean ± standard error of the mean of three independent experiments and $p<0.05$ was considered to be statistically significant.

3. Discussion

The first series of analogs exhibited a VEGF-A_{165}/NRP-1 inhibition which did not exceed 40%, except for compound **17**. The effect of the linker length of separating both guanidium functions was difficult to evaluate, since compound **15** inhibited very weakly VEGF-A_{165}/NRP-1 binding (entry 2, Table 1), while a modest activity was observed for **16** and **17** at 100 µM concentration (entries 3-4). Compound **27**, based on sugar scaffold B and functionalized in the same manner as compound **1** was less active than this latter, suggesting a non-adapted spatial disposition of crucial residues (entry 5). Compounds **34**, **33** and the corresponding diols **38** and **37** showed a weak affinity for NRP-1, suggesting that triazole moiety could be too rigid linker and deleterious for binding (entries 6-9).

The second series showed general binding inhibition properties higher than 40% at 100 µM, excepted for compound **63**. Arginine anchored at C1 was beneficial for NRP-1 binding, as shown for compound **50** and **56** (entries 12-13). Compound **56** based on scaffold B showed a 67% binding inhibition at 100 µM and an IC_{50} of 39 µM, this result being in agreement with *in silico* predictions (see 2.2). However, the presence of amino-methyl and hydroxy-carboxymethyl groups recommended by molecular modeling was not beneficial for binding (entries 14-15), suggesting that the added chains were not long enough. Compound **69** obtained by conjugation with the 6-fluoroglycoside **68** displayed a 70% binding inhibition at 100µM and an IC_{50} of 70 µM, pointing out that the presence of the sugar derivative was not deleterious for NRP-1 binding.

The biological properties of the compounds having the higher affinity were evaluated on HUVECs firstly through the downstream signaling pathways of NRP-1. All the compounds induced a similar change of ERK1/2 signaling pathways, which did not really impact the viability of endothelial cells even with a high concentration of 200 µM. Nevertheless, some compounds showed a significant effect on angiogenesis network formation. Unexpectedly, while compound **69** did not have the best affinity (IC_{50} = 69 µM), this latter demonstrated the most efficient properties toward inhibition of tubules formations. On the contrary, compound **56**, the most affine compound (IC_{50} = 39µM), showed a lack of anti-angiogenic effect, which could be explained by the significant increase of AKT phosphorylation with this compound (ratio 2.52). Indeed, AKT signaling pathway is well known to induce cell survival that limit the endothelial cell network remodeling during angiogenic process. The complexation of NRP-1 with its two well-known co-receptors, VEGFR and plexin (receptor of semaphorins), which have opposite effect on endothelial cell migration and tubulogenesis,[46] could explain the results obtained with the different compounds. Indeed, the binding domains for semaphorin (a1/a2) are relatively close to the VEGF-A_{165} binding domains (b1/b2) and some compounds could also interact partially with a1/a2 semaphorin domains. Compounds **50, 56** and **61** which induced a slight invasive effect, could modify the signaling pathway involving the plexin/semaphorine/NRP-1 ternary complex. On the contrary, compound **69** seems to be more specific of the b1/b2 domains of VEGF due to its similar effect to tuftsin and to a lesser extent to bevacizumab. Consequently, despite a good affinity for NRP-1 attested by decrease of MAPK-induced activation, an evaluation of all

signaling pathways regulated by NRP-1[47] could allow elucidation of the different cellular effects observed with the tested compounds.

4. Conclusion

We have synthesized two series of carbohydrate-based peptidomimetics targeting NRP-1. The design of these ligands was based on rational modification of compound **1** to enhance binding interaction. Compound **56** inhibited VEGF-A_{165}/NRP-1 binding with an IC_{50} = 39 µM, which is in the range of activity described by others working on NRP-1 inhibitors. Compound **56** demonstrated specificity for NRP-1 over VEGF-R2 binding more than 10-fold. No cytotoxic effects on HUVECs proliferation or viability were measured for all tested compounds. Interestingly, compound **69** (IC_{50} = 69µM) demonstrated a significant effect on tubules formation which could be compared to tuftsin and to a lesser extent to bevacizumab without promoting the endothelial cells migration. In addition to its use as a potential anti-angiogenic compound, this compound could be envisaged to target cells overexpressing NRP-1 as cancer cells.

5. Experimental section

5.1. Chemistry

Reagents, general methods, experimental procedures for synthesis and characterizations of all intermediates are given in SI. Compounds **2-6** have been prepared according to literature procedures.[25]

N$^{\alpha}$-[2-C-[2,3-O-(1-methylethylidene)-5-benzosulfonylamino-α-D-galactofuranosyl]carbonymethyl)]-L-arginine methyl ester 50

To a solution of **49** (500 mg, 0.85 mmol) in THF (20 mL) and water (3 mL) was added LiOH (65 mg, 2.55 mmol, 3 eq.) and the mixture was stirred until completion of the reaction monitored by tlc. Amberlite® IR-120 was added until pH = 3-4 and the mixture was filtered off. The solvent were evaporated and the obtained carboxylic acid was solubilized in MeOH (20 mL) and Pd/C (10%) (40% w/w) was added. After stirring under H_2 atmosphere (40 psi) until completion of the reaction, the mixture was filtered through a pad of celite and the filtrate was concentrated *in vacuo*. The crude was purified by column chromatography (C-18 silica gel, H_2O/MeOH) and lyophilized to provide **50** (367 mg, 82%). White solid. *Rf;* 0.24 (H_2O/MeOH: 1/1). HPLC (70/30): t_R = 6.049 min. $[\alpha]_D$= +15.0° (c = 0.2, H_2O). ^{1}H NMR (400 MHz, D_2O): δ = 1.22 (s, 3H, C(C*H*$_3$)$_2$), 1.33 (s, 3H, C(C*H*$_3$)$_2$), 1.49-1.56 (m, 2H, C*H*$_2$ *arg*), 1.64 (m, 1H, C*H*$_2$ *arg*), 1.77 (m, 1H, C*H*$_2$ *arg*), 2.53-2.63 (m, 2H, *H*-2), 3.07-3.11 (m, 3H, *H*-7, 2 C*H*$_2$ *arg*), 3.18 (dd, 1H, J_{gem} = 13.5, $J_{6,7'}$ = 4.5 Hz, *H*-7'), 3.60 (m, 1H, *H*-6), 3.85 (td, 1H, $J_{3,4}$ = 2.5 Hz, *H*-3), 4.12 (dd, 1H, $J_{H\text{-}\alpha,A1}$ = 8.0 Hz, $J_{H\text{-}\alpha,A1'}$ = 5.0 Hz, *H*-α), 4.67-4.69 (m, 2H, *H*-4, *H*-5), 7.53-7.57 (m, 2H, *H*-Ar), 7.64 (m, 1H, *H*-Ar), 7.79-7.82 (m, 2H, *H*-Ar). ^{13}C NMR (100 MHz, D_2O): δ = 23.6 (*C*H$_3$), 24.4 (*C*H$_2$ *arg*), 24.7 (*C*H$_3$), 28.8 (*C*H$_2$ *arg*), 34.7 (*C*-2), 40.6 (*C*H$_2$ *arg*), 41.3 (*C*-7), 54.8 (*C*-α), 77.7 (*C*-3), 79.1 (*C*-6), 80.3 (*C*-5), 81.1 (*C*-4), 112.8 (*C*(CH$_3$)$_2$), 126.7 (2 *C*-Ar), 129.5 (2 *C*-Ar), 133.6 (*C*-Ar), 137.9 (*C*-Ar), 156.7 (*C*=N), 171.9 (*C*=O), 178.4 (*C*=O). IR (pellets) υ: 3360, 3180, 2990, 2943, 2876, 1645, 1586. ESI-HRMS: m/z calcd for $C_{22}H_{33}N_5O_8SNa$ $[M+Na]^+$ 550.1942; found 550.1956.

5.1.1. N$^{\alpha}$-[2-*C*-[2,3-*O*-(1-methylethylidene)-5-benzosulfonylamino-α-D-ribofuranosyl]carbonylmethyl)]-L-arginine methyl ester 56

Prepared starting from **55** (150 mg, 0.26 mmol) following procedure described for **50** Yield: 70% (94 mg). White solid. *Rf;* 0.22 (H_2O/MeOH: 1/1). HPLC (70/30): t_R = 5.526 min. $[\alpha]_D$= +17.1° (c = 0.1, H_2O). ^{1}H NMR (250 MHz, D_2O): δ = 1.37 (s, 3H, C(C*H*$_3$)$_2$), 1.52 (s, 3H, C(C*H*$_3$)$_2$), 1.59-1.95 (m, 4H, C*H*$_2$ *arg*), 2.63 (dd, 1H, J_{gem} = 14.5, $J_{2,3}$ = 7.5 Hz, *H*-2), 2.72 (dd, 1H, $J_{2,3'}$ = 6.5 Hz, *H*-2'), 3.06 (dd, 1H, J_{gem} = 14.0, $J_{6,7}$ = 8.0 Hz, *H*-7), 3.14 (dd, 1H, $J_{6,7'}$ = 6.0 Hz, *H*-7'), 3.23 (t, 2H, *J* = 7.0 Hz, C*H*$_2$ *arg*), 4.12 (dd, 1H, *H*-6), 4.20-4.27 (m, 2H, *H*-3, *H*-α), 4.74 (d, 1H, $J_{4,5}$ = 6.0 Hz, *H*-5), 4.84 (dd, 1H, $J_{3,4}$ = 4.0 Hz, *H*-4), 7.66-7.80 (m, 3H, *H*-Ar), 7.91-7.95 (m, 2H, *H*-Ar). ^{13}C NMR (62.9 MHz, D_2O): δ = 23.6, 24.4, 25.0 (2 *C*H$_3$, *C*H$_2$ *arg*), 28.8 (*C*H$_2$ *arg*), 35.2 (*C*-2), 40.7 (*C*H$_2$ *arg*), 41.8 (*C*-7), 54.6 (*C*-α), 76.6 (*C*-3), 80.7, 82.1, 82.4 (*C*-4, *C*-5, *C*-6), 113.1 (*C*(CH$_3$)$_2$), 126.6 (2 *C*-Ar), 129.6 (2 *C*-Ar), 133.6 (*C*-Ar), 138.3 (*C*-Ar), 156.7 (*C*=N), 171.7 (*C*=O), 178.5 (*C*=O). IR (pellets) υ: 3331, 3134, 2988, 2928, 2860, 1632, 1575, 1446. ESI-HRMS: m/z calcd for $C_{22}H_{33}N_5O_8SNa$ $[M+Na]^+$ 550.1942; found 550.1957.

N$^{\alpha}$-[2-*C*-[2,3-*O*-(1-methylethylidene)-5-[((4-aminomethyl)benzo)sulfonylamino]-α-D-ribofuranosyl]carbonymethyl)]]-L-arginine methyl ester 61

To a solution of **60** (80 mg, 0.14 mmol) in MeOH (5 mL), was added Pd/C (10%) (8 mg, 10% w/w). After stirring under H_2 atmosphere (15 psi) until completion of the reaction, the mixture was filtered through a pad of celite and the filtrate was concentrated *in vacuo*. The crude was purified by column chromatography (C-18 silica gel, H_2O/MeOH) and lyophilized to provide **61** (65 mg, 87%). White solid. *Rf;* 0.38 (H_2O/CH_3CN/TFA: 7/3/0.1%). HPLC (70/30): t_R = 3.681 min. $[\alpha]_D$= +6.9° (c = 0.1, H_2O). ^{1}H NMR (400 MHz, DMSO-d_6): δ = 1.25 (s, 3H, C(C*H*$_3$)$_2$), 1.38 (s, 3H, C(C*H*$_3$)$_2$), 1.45-1.61 (m, 3H, C*H*$_2$ *arg*), 1.73 (m, 1H, C*H*$_2$ *arg*), 2.47-2.53 (m, 2H, 2 *H*-2), 2.68 (m, 1H, *H*-7), 2.82 (m, 1H, *H*-7'), 3.07-3.11 (m, 2H, C*H*$_2$ *arg*), 3.87 (t, 1H, $J_{6,7}$ = 7.0 Hz, *H*-6), 4.10-4.17 (m, 3H, *H*-3, C*H*$_2$-NH$_2$), 4.20 (td, 1H, *J* = 8.0, *J* = 5.0 Hz, *H*-α), 4.63 (d, 1H, $J_{4,5}$ = 6.0 Hz, *H*-5), 4.66 (dd, 1H, $J_{3,4}$ = 3.5 Hz, *H*-4), 6.99 & 7.20 (br s, 4H, 4 N*H*), 7.59 (t, 1H, *J* = 5.5 Hz, N*H*), 7.67 (d, 2H, *J* = 8.0 Hz, 2 *H*-Ar), 7.84-7.90 (m, 3H, 2 *H*-Ar, N*H*), 8.18 (d, 1H, $J_{NH,\alpha}$ = 8.0 Hz, N*H*), 8.28 (br s, 3H, C*H*$_2$-NH$_3^+$). ^{13}C NMR (100 MHz, DMSO-d_6): δ = 25.2 (*C*H$_3$), 25.5 (*C*H$_2$ *arg*), 26.6 (*C*H$_3$), 28.8 (*C*H$_2$ *arg*), 35.3 (*C*-2), 40.7 (*C*H$_2$ *arg*), 42.2 (*C*-7), 42.9 (*C*H$_2$-NH$_2$), 51.8 (*C*-α), 77.0 (*C*-3), 81.3 (*C*-4), 82.1 (*C*-6), 83.0 (*C*-5), 111.8

(*C*(CH_3)$_2$), 127.3 (2 *C*-Ar), 130.1 (2 *C*-Ar), 139.0 (*C*-Ar), 140.7 (*C*-Ar), 157.1 (*C*=N), 158.5 (TFA), 170.0 (*C*=O amide), 173.9 (*C*=O acid). IR (pellets) υ: 3393, 3189, 2990, 2928, 1676, 1546. ESI-HRMS: m/z calcd for $C_{23}H_{37}N_6O_8S$ $[M+Na]^+$ 557.2388; found 557.2399.

5.1.2. Compound 69

To a solution of alcyne **67** (33 mg, 0.04 mmol, 1 eq.) and azido sugar **68** (15 mg, 0.04 mmol, 1 eq.) in a water/*t*BuOH mixture (0.5 mL/0.5 mL) were added sodium ascorbate (2.4 mg, 0.008 mmol, 0.2 eq.) and $Cu(OAc)_2$ (1.2 mg, 0.004 mmol, 0.1 eq.). The solution turned progressively pale green and the mixture was stirred at room temperature until completion of the reaction (the blue color reappeared). Chelex® resin (100 mg) was then added to the solution and the suspension was stirred until the solution became colorless. The resin was filtered off and the solvent were removed under reduced pressure. The crude was purified by column chromatography (C-18 silica gel, H_2O/MeOH) and lyophilized to provide **69** (37 mg, 77%).White solid. *Rf;* 0.29 (H_2O/MeOH: 1/1). HPLC (70/30): t_R = 4.180 min. $[\alpha]_D$= -6.7° (c = 0.1, H_2O). ^{1}H NMR (400 MHz, D_2O): δ = 1.31 (s, 3H, C(C*H*$_3$)$_2$), 1.46 (s, 3H, C(C*H*$_3$)$_2$), 1.53-1.61 (m, 2H, C*H*$_2$ *arg*), 1.69 (m, 1H, C*H*$_2$ *arg*), 1.84 (m, 1H, C*H*$_2$ *arg*), 2.59 (dd, 1H, J_{gem} = 15.0, $J_{2,3}$ = 8.0 Hz, *H*-2), 2.66 (dd, 1H, $J_{2',3}$ = 6.5 Hz, *H*-2'), 3.04 (dd, 1H, J_{gem} = 13.5, $J_{6,7}$ = 8.5 Hz, *H*-7), 3.08-3.17 (m, 3H, C*H*$_2$ *arg*, *H*-7'), 3.26 (m, 1H, *H*-11), 3.42- 3.59 (m, 3H, *H*-12, *H*-13, *H*-14), 4.08 (dd, 1H, $J_{6,7}$ = 8.5, $J_{6,7'}$ = 6.5 Hz, *H*-6), 4.14-4.25 (m, 3H, *H*-3, *H*-α, *H*-9), 4.33 (m, 1H, *H*-9'), 4.47 (d, 1H, $J_{10,11}$ = 8.0 Hz, *H*-10), 4.61 (dd, 2H, $J_{H,F}$ = 47.5, $J_{14,15}$ = 2.0 Hz, *H*-15), 4.68 (d, 1H, $J_{4,5}$ = 6.0 Hz, *H*-5), 4.73-4.77 (m, 3H, *H*-4, 2 *H*-8), 7.94 (d app., 2H, *J* = 8.0 Hz, *H*-Ar), 7.99 (d app., 2H, *H*-Ar), 8.49 (*H*-triazole). ^{13}C NMR (62.9 MHz, D_2O): δ = 23.9 (*C*H_3), 24.6 (*C*H$_2$ *arg*), 25.3 (*C*H_3), 29.1 (*C*H$_2$ *arg*), 35.6 (*C*-2), 40.9 (*C*H$_2$ *arg*), 42.2 (*C*-7), 50.9 (*C*-8), 54.8 (*C*-α), 68.5 (d, $J_{C,F}$ = 7.0 Hz, *C*-13), 68.7 (*C*-9), 73.1 (*C*-11), 74.5 (d, $J_{C,F}$ = 18.0 Hz, *C*-14), 75.7 (*C*-12), 77.0 (*C*-3), 81.0, 82.4, 82.7 (*C*-4, *C*-5, *C*-6), 82.1 (d, $J_{C,F}$ = 168.0 Hz, *C*-15), 102.9 (*C*-10), 113.3 (*C*(CH_3)$_2$), 124.4 (*C*-Ar), 126.7 (2 *C*-Ar), 127.8 (2 *C*-Ar), 134.7 (*C*-triazole), 138.5 (*C*-Ar), 146.0 (*C*-triazole), 156.9 (*C*=N), 172.0 (*C*=O), 178.7 (*C*=O) ^{19}F NMR (235.2 MHz, D_2O): δ = -235.16 (td, $J_{H,F}$ = 47.0, $J_{H,F}$ = 27.0 Hz). IR (ATR) υ: 3346, 3236, 1655, 1505, 1150. ESI-HRMS: m/z calcd for $C_{32}H_{47}FN_8O_{13}SNa$ $[M+Na]^+$ 825.2860; found 825.2845.

5.2. Molecular modeling

The structures of the investigated compounds were prepared according to the CORINA[48] (for the 3D conformers) and MOPAC[49] softwares (for the atomic charges). The chemicals were firstly docked using the GOLD software (see SI for details).[50] The protein target was the 3D structure of the b1 domain of NRP-1 as solved by X-ray when bound with the small molecule EG00229[26] (pdb code 3I97).

5.3. Biocrystallography

A crystallization screening of NRP-1-b1 fragment including a 6-His tag at the *N*-terminus was carried out in presence of compounds **27**, **40**, **50** and **56** (see SI for details). The crystal structure has been deposited at the Protein Data Bank[51] with code 5C7G.

5.4. Biological Assay

General procedure for receptor binding assays: The binding of new derivatives to recombinant NRP-1 protein was determined using a competition assay initially described by Tirand *et al.* (see details in SI).[43]

General procedure for HUVECs culture and treatments: HUVECs were collected from umbilical cords as previously described by Jaffe *et al.* (see details in SI).[52]

General procedure for NRP1 signaling pathways: For analysis of pAKT and pERK1/2 expression, western blotting was realized as previously described (see details in SI).[53]

General procedure for cell viability assays: Roche's WST-1 cell proliferation reagent is a simple, colorimetric assay designed to measure the relative proliferation rates of cells in culture (see details in SI).

Angiogenesis assay: HUVECs were plated (90,000 cells/cm2) onto 24-well plate ibidi precoated with Matrigel™ Basement Membrane Matrix (BD Biosciences, France, see details in SI).

Acknowledgments

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Supplementary Material

Experimental procedures for synthesis and characterizations of all intermediates and copies of ^{1}H and ^{13C} NMR spectra, crystallographic summary of NRP-1/bicine structure, protein production and purification, biological assays and experimental procedures for molecular modeling and biocrystallography. This material is available free of charge via the Internet at

Abbreviations

MRI, magnetic resonance imaging; AKT, serine/threonine protein kinase; ERK1/2, extracellular signal-regulated protein kinases 1 and 2.

Supporting information

Carbohydrate-based peptidomimetics targeting neuropilin-1: synthesis, molecular docking study and biological activities

*Mylène Richard, Alicia Chateau, Christian Jelsch, Claude Didierjean, Xavier Manival, Christophe Charron, Bernard Maigret, Muriel Barberi-Heyob, Yves Chapleur, Cédric Boura, Nadia Pellegrini-Moïse**

Experimental procedures for:

Synthesis and characterizations of all compounds
Molecular modeling
Protein production, purification and biocrystallograhy
Biological assays

Table and Figures:

Copies of ^{1}H and ^{13C}NMR spectra of compounds **7-67, 69**

Experimental procedures and characterizations of all compounds

Reagents and general methods

DMF was dried by distillation from calcium hydride. Other solvents and reagents were purchased from commercial sources and used without further purification. TLC analyses were performed using standard procedures on Kieselgel 60 F254 plates (Merck). Compounds were visualized using UV light (254 nm) and 30% methanolic H_2SO_4/heat as developing agent. Column chromatography was performed on silica gel SI 60 (63-200 µm) or Lichroprep RP-18 (40-63µm) (Merck). FTIR spectra were recorded on a Perkin-Elmer spectrum 1000 on NaCl windows (film) or KBr pellets (cm^{-1}). Melting points were determined with a Tottoli apparatus and are uncorrected. Optical rotations were measured on an Anton Paar MC300 polarimeter. ^1H and ^{13}C NMR spectra were recorded on a Bruker spectrometer DPX250 (250 MHz and 62.9 MHz, respectively) and DRX400 (400 MHz and 100.6 MHz, respectively). For complete assignment of ^1H and ^{13}C signals, two-dimensional ^1H,^1H COSY and ^1H,^{13}C correlation spectra were recorded. Mass spectra (MS) were recorded on a ESI/QqTOF Bruker spectrometer. Purity of the compounds for biological assays was confirmed to be greater than 95% by high-performance liquid chromatography. HPLC analyses were run on a Waters system (2695 ebpump, auto sampler injector, 2998 PDA detector and 2424 ELSD detector) controlled by the Empower software. Analyses were performed on a Platinium C18 (5 µm, 250x4.6 mm) from Grace with a AcCN/H_2O/0.2%TFA mixture (proportions given in brackets) at 1 mL/min.

3,6-Anhydro-2-deoxy-*N*-[2-(1*H*-indol-3-yl)ethyl]-4,5-*O*-(1-methylethylidene)-7-hydroxy-L-*galacto*-heptonamide 7

To a stirred solution of **6** (3.73 g, 8.40 mmol) in methanol (180 mL) at 0°C was added dropwise an aqueous solution of 1N HCl (60 mL). After stirring at room temperature until completion of the reaction, sat. aq. $NaHCO_3$ was added until pH = 7. Half of the solvent was removed under *vacuum* and the product was extracted with CH_2Cl_2 (3 x 100 mL). The organic layer was dried over $MgSO_4$, filtered and the solvent was evaporated. To a stirred solution of this diol in methanol (180 mL) was added $NaIO_4$ (3.59 g, 16.8 mmol, 2 eq.) under argon. After stirring at room temperature until completion of the reaction, the solvent was removed by half *in vacuo*. The mixture was diluted with CH_2Cl_2 (200 mL). The organic layer was washed with water (3 x 75 mL), dried over $MgSO_4$, filtered and the solvent was removed under reduced pressure. The product was used without further purification.To a stirred solution of the obtained aldehyde in MeOH (190 mL) was added $NaBH_4$ (320 mg, 8.40 mmol, 1 eq.) at 0°C. After 1 h at room temperature, the solvent was removed under reduced pressure. The residue was diluted in CH_2Cl_2 (100 mL) and the organic layer was washed with a solution of 1N HCl (30 mL) and with water until pH = 7. The organic layer was dried over $MgSO_4$, filtrered and evaporated under reduced pressure. The crude product **7** (2.26 g, 72%) was used without further purification. Yellow powder. *Rf;* 0.38 (CH_2Cl_2/MeOH 9/1). $[\alpha]_D$= +3.0 (c = 1.50, $CHCl_3$). Mp. = 94°C. ^1H NMR (250 MHz, $CDCl_3$): δ = 1.29 (s, 3H, C(C*H*$_3$)$_2$), 1.45 (s, 3H, C(C*H*$_3$)$_2$), 2.53-2.68 (m, 3H, 2 *H*-2, O*H*), 3.00 (t, 2H, $J_{\alpha,\beta}$ = 6.5 Hz, 2 *H*-β), 3.51-3.71 (m, 3H, *H*-6, 2 *H*-α), 3.83-3.89 (m, 3H, *H*-3, 2 *H*-7), 4.60 (dd, 1H, $J_{4,5}$ = 6.0, $J_{5,6}$ = 3.5 Hz, *H*-5), 4.70 (dd, 1H, $J_{3,4}$ = 3.5 Hz, *H*-4), 6.22 (br s, 1H, N*H*amide), 7.09-7.26 (m, 3H, 3 *H*-Ar), 7.41 (d, 1H, *J* = 8.0, *H*-Ar), 7.63 (d, 1H, *J* = 8.0, *H*-Ar), 8.55 (br s, 1H, N*H*indole). ^{13}C NMR (62.9 MHz, $CDCl_3$): δ = 23.4 (*C*H$_3$), 23.9 (*C*H$_3$), 24.7 (*C*-β), 35.1 (*C*-2), 38.6 (*C*-α), 59.8 (*C*-7), 76.9 (*C*-3), 80.1, 80.2, 80.5 (*C*-4, *C*-5, *C*-6), 110.2, 111.4, 111.6 (2 *C*-Ar, *C*(CH$_3$)$_2$), 117.6 (*C*-Ar), 118.3 (*C*-Ar), 120.9 (*C*-Ar), 121.4 (*C*-Ar), 126.3 (*C*-Ar), 135.3 (*C*-Ar), 169.5 (*C*=O). IR (film) υ: 3327, 1645. ESI-HRMS: m/z calcd for $C_{20}H_{27}N_2O_5$ $[M+H]^+$ 375.1914; found 375.1898.

3,6-Anhydro-2-deoxy-*N*-[2-(1*H*-indol-3-yl)ethyl]-4,5-*O*-(1-methylethylidene)-7-(4-methylbenzenesulfonate)-L-*galacto*-heptonamide 8

To a solution of **7** (1.94 g, 5.2 mmol) in CH_2Cl_2 (50 mL) were added Et_3N (1.8 mL, 13 mmol, 2.5 eq.), DMAP (61 mg, 0.5 mmol, 0.1 eq.) and tosyl chloride (2 g, 10.4 mmol, 2 eq.) and the mixture was stirred for 24 h at room temperature. The solution was washed with 1N HCl (20 mL), sat. aq. $NaHCO_3$ (20 mL) and brine (20 mL). The organic layer was dried over $MgSO_4$, filtered and the solvent was evaporated *in vacuo*. The compound was purified by flash chromatography (silica gel, cyH/EA) to provide **8** (2.47 g, 90%). White solid. *Rf;* 0.49 (CH_2Cl_2/MeOH 9/1). $[\alpha]_D$= +6.0 (c = 1.0, $CHCl_3$). Mp = 64°C. ^{1}H NMR (400 MHz, $CDCl_3$): δ = 1.21 (s, 3H, C*H*$_3$), 1.30 (s, 3H, C*H*$_3$), 2.44 (s, 3H, Ph-C*H*$_3$), 2.49-2.51 (m, 2H, *H*-2), 2.98 (t, 2H, $J_{\alpha,\beta}$ = 6.5 Hz, *H*-β), 3.49 (m, 1H, *H*-α), 3.62 (m, 1H, *H*-6), 3.68-3.77 (m, 2H, *H*α, *H*-3), 4.12 (dd, 1H, $J_{6,7}$ = 7.5, $J_{7,7'}$ = 11.0 Hz, *H*-7), 4.25 (dd, 1H, $J_{6,7'}$ = 4.0 Hz, *H*-7'), 4.54 (dd, 1H, $J_{3,4}$ = 3.5, $J_{4,5}$ = 6.0 Hz, *H*-4), 4.59 (dd, 1H, $J_{5,6}$ = 4.0 Hz, *H*-5), 6.13 (t, 1H, *J* = 5.5 Hz, CON*H*), 7.08-7.12 (m, 2H, *H*-Ar), 7.19 (m, 1H, *H*-Ar), 7.33 (d, 2H, *J* = 8.0, *H*-Ar), 7.41(d, 1H, *J* = 8.0, *H*-Ar), 7.59 (d, 1H, *J* = 8.0, *H*-Ar), 7.79 (d, 2H, *J* = 8.5 Hz, *H*-Ar), 8.58 (s, 1H, N*H*-Ar). ^{13}C NMR (100 MHz, $CDCl_3$): δ = 21.7 (*C*H$_3$-Ph), 24.6 (*C*H$_3$), 24.9 (*C*-β), 25.7 (*C*H$_3$), 36.4 (*C*-2), 39.6 (*C*-α), 68.2 (*C*-7), 78.5 (*C*-3), 79.9 (*C*-6), 80.4 (*C*-5), 81.4 (*C*-4), 111.4 (*C*-Ar), 112.6 (*C*(CH$_3$)$_2$), 112.8 (*C*-Ar), 118.6 (*C*-Ar), 119.2 (*C*-Ar), 122.0 (*C*-Ar), 122.5 (*C*-Ar), 127.4 (*C*-Ar), 128.0 (2 *C*-Ar), 129.9 (2 *C*-Ar), 132.5 (*C*-Ar), 136.5 (*C*-Ar), 145.2 (*C*-Ar), 170.3 (*C*=O). IR (film) υ: 1654, 1535. ESI-HRMS: m/z calcd for $C_{27}H_{32}N_2O_7SNa$ $[M+Na]^+$ 551.1822; found 551.1852.

3,6-Anhydro-2,7-dideoxy-*N*-[2-(1*H*-indol-3-yl)ethyl]-4,5-*O*-(1-methylethylidene)-7-[[3-[[(phenylmethoxy)carbonyl]amino]propyl]amino]-L-*galacto*-heptonamide 9

A solution of **8** (422 mg, 0.8 mmol, 1 eq.) and amine (0.8 mmol, 4 eq.) in isopropanol (4 mL) was heated at 100°C for 48h in a sealed tube. The solvent was removed under reduced pressure and the residue was diluted with EtOAc (10 mL) and washed with sat. aq. $NaHCO_3$ (2x5 mL). The organic layer was dried over $MgSO_4$, filtered and the solvent was evaporated under reduced pressure. The compound was purified by flash chromatography (silica gel, cyH/EA) to provide **9** (324 mg, 72%). Colorless gum. *Rf;* 0.33 (CH_2Cl_2/MeOH 9/1). $[\alpha]_D$= +10.3 (c = 0.5, $CHCl_3$). ^{1}H NMR (250 MHz, $CDCl_3$): δ = 1.27 (s, 3H, C*H*$_3$), 1.42 (s, 3H, C*H*$_3$), 1.59-1.76 (m, 2H, *H*-9), 2.55-2.59 (m, 2H, *H*-2), 2.69-2.74 (m, 2H, *H*-10), 2.77-2.92 (m, 2H, *H*-7), 2.99 (t, 2H, $J_{\alpha,\beta}$ = 7.5 Hz, *H*-β), 3.13-3.23 (m, 2H, *H*-8), 3.44-3.79 (m, 4H, *H*-3, *H*-6, *H*-α), 4.55 (dd, 1H, $J_{4,5}$ = 6.0, $J_{5,6}$ = 3.5 Hz, *H*-5), 4.60 (dd, 1H, $J_{3,4}$ = 3.5 Hz, *H*-4), 5.12 (s, 2H, C*H*$_2$-Ph), 5.50 (br s, 1H, NH), 6.29 (br s, 1H, N*H*), 7.05-7.23 (m, 3H, *H*-Ar), 7.32-7.42 (m, 6H, *H*-Ar), 7.61 (d, 1H, *J* = 8.0 Hz, *H*-Ar), 8.81 (br s, 1H, N*H*). ^{13}C NMR (62.9 MHz, $CDCl_3$): δ = 24.7 (*C*H$_3$), 25.1 (*C*-β), 25.8 (*C*H$_3$), 29.2 (*C*-9), 36.4 (*C*-2), 39.5 (*C*-α), 39.7 (*C*-8), 47.6 (*C*-7), 47.9 (*C*-10), 66.7 (*C*H$_2$-Ph), 78.1 (*C*-3), 80.4 (*C*-6), 81.0 (*C*-4), 81.5 (*C*-5), 111.4 (*C*-Ar), 112.3 (*C*(CH$_3$)$_2$), 112.9 (*C*-Ar), 118.7 (*C*-Ar), 119.3 (*C*-Ar), 122.0 (*C*-Ar), 122.2 (*C*-Ar), 127.5 (*C*-Ar), 128.1 (2 *C*-Ar), 128.5 (3 *C*-Ar), 136.4 (*C*-Ar), 136.6 (*C*-Ar), 156.8 (O*C*ONH), 170.5 (*C*ONH). IR (film) υ: 3312, 1714, 1645. ESI-HRMS: m/z calcd for $C_{31}H_{40}N_4O_6Na$ $[M+Na]^+$ 587.2840; found 587.2866.

3,6-Anhydro-2,7-dideoxy-*N*-[2-(1*H*-indol-3-yl)ethyl]-4,5-*O*-(1-methylethylidene)-7-[[4-[[(phenylmethoxy)carbonyl]amino]butyl]amino]-L-*galacto*-heptonamide 10

Prepared starting from **8** (422 mg, 0.8 mmol) following procedure described for **9**. Yield: 70% (323 mg). Colorless gum. *Rf;* 0.29 (CH_2Cl_2/MeOH 9/1). $[\alpha]_D$= +9.1 (c = 1.0, $CHCl_3$). ^{1}H NMR (400 MHz, $CDCl_3$): δ = 1.26 (s, 3H, C*H*$_3$), 1.41 (s, 3H, C*H*$_3$), 1.50-1.56 (m, 4H, *H*-9, *H*-10), 2.55 (dd, 2H, J_{gem}= 6.5 Hz, $J_{2,3}$ = 4.0 Hz, $J_{2',3}$ = 2.0 Hz, *H*-2), 2.61-2.65 (m, 2H, *H*-11), 2.81 (dd, 1H, J_{gem} = 12.5 Hz, $J_{6,7}$ = 7.5 Hz, H-7), 2.88 (dd, 1H, $J_{6,7'}$ = 4.5 Hz, H-7'), 2.98 (t, 2H, $J_{\alpha,\beta}$ = 6.5 Hz, *H*-β), 3.17-3.21 (m, 2H, *H*-8), 3.52-3.61 (m, 2H, *H*-α, *H*-6), 3.65 (m, 1H, *H*-α), 3.74

(m, 1H, *H*-3), 4.49-4.55 (m, 2H, *H*-4, *H*-5), 5.10 (s, 2H, C*H*$_2$-Ph), 5.47 (br s, 1H, N*H*), 6.26 (t, 1H, *J* = 5.5 Hz, N*H*), 7.05 (s, 1H, *H*-Ar), 7.12 (m, 1H, *H*-Ar), 7.20 (m, 1H, *H*-Ar), 7.31-7.40 (m, 6H, *H*-Ar), 7.61 (d, 1H, *J* = 8.0 Hz, *H*-Ar), 8.72 (br s, 1H, N*H*). ^{13}C NMR (100 MHz, $CDCl_3$): δ = 24.7 (*C*H$_3$), 25.1 (*C*-β), 25.8 (*C*H$_3$), 26.6, 27.7 (*C*-9, *C*-10), 36.3 (*C*-2), 39.7 (*C*-α), 40.8 (*C*-8), 47.9 (*C*-7), 49.4 (*C*-11), 66.6 (*C*H$_2$-Ph), 78.0 (*C*-3), 80.2 (*C*-6), 81.0, 81.5 (*C*-4, *C*-5), 111.4 (*C*-Ar), 112.3 (*C*(CH$_3$)$_2$), 112.8 (*C*-Ar), 118.7 (*C*-Ar), 119.3 (*C*-Ar), 122.0 (*C*-Ar), 122.3 (*C*-Ar), 127.4 (*C*-Ar), 128.2 (2 *C*-Ar), 128.5 (3 *C*-Ar), 136.4 (*C*-Ar), 136.7 (*C*-Ar), 156.6 (O*C*ONH), 170.5 (*C*ONH). IR (film) υ: 3312, 1716, 1648. ESI-HRMS: m/z calcd for $C_{32}H_{42}N_4O_6Na$ $[M+Na]^+$ 601.2997; found 601.3002.

3,6-Anhydro-2,7-dideoxy-*N*-[2-(1*H*-indol-3-yl)ethyl]-4,5-*O*-(1-methylethylidene)-7-[[5-[[(phenylmethoxy)carbonyl]amino]pentyl]amino]-L-*galacto*-heptonamide 11

Prepared starting from **8** (422 mg, 0.8 mmol) following procedure described for **9**. Yield: 80% (378 mg). Colorless gum. *Rf;* 0.37 (CH_2Cl_2/MeOH 9/1). $[\alpha]_D$= +5.5 (c = 1.0, $CHCl_3$). ^{1}H NMR (250 MHz, $CDCl_3$): δ = 1.27 (s, 3H, C*H*$_3$), 1.43 (s, 3H, C*H*$_3$), 1.29-1.53 (m, 6H, *H*-9, *H*-10, *H*-11), 2.57 (d, 2H, $J_{2,3}$ = 6.5 Hz, *H*-2), 2.62 (t, 2H, $J_{11,12}$ = 7.0 Hz, *H*-12), 2.82-2.92 (m, 2H, *H*-7), 2.98 (t, 2H, $J_{\alpha,\beta}$ = 7.5 Hz, *H*-β), 3.16-3.21 (m, 2H, *H*-8), 3.54-3.65 (m, 3H, *H*-6, *H*-α), 3.81 (m, 1H, *H*-3), 4.56 (dd, 1H, $J_{4,5}$ = 6.0, $J_{5,6}$ = 3.5 Hz, *H*-5), 4.61 (dd, 1H, $J_{3,4}$ = 3.5 Hz, *H*-4), 4.92 (br s, 1H, N*H*), 5.11 (s, 2H, C*H*$_2$-Ph), 6.16 (t, 1H, *J* = 5.5 Hz, N*H*), 7.05 (s, 1H, *H*-Ar), 7.12 (m, 1H, *H*-Ar), 7.20 (m, 1H, *H*-Ar), 7.31-7.39 (m, 6H, *H*-Ar), 7.62 (d, 1H, *J* = 8.0 Hz, *H*-Ar), 8.78 (br s, 1H, N*H*). ^{13}C NMR (62.9 MHz, $CDCl_3$): δ = 24.2 (*C*-10), 24.7 (C*H*$_3$), 25.2 (*C*-β), 25.9 (C*H*$_3$), 29.2, 29.7 (*C*-9, *C*-11), 36.3 (*C*-2), 39.7 (*C*-α), 40.9 (*C*-8), 48.0 (*C*-7), 49.7 (*C*-12), 66.6 (*C*H$_2$-Ph), 78.0 (*C*-3), 80.5 (*C*-6), 81.1 (*C*-4), 81.5 (*C*-5), 111.3 (*C*-Ar), 112.3 (*C*(CH$_3$)$_2$), 112.8 (*C*-Ar), 118.7 (*C*-Ar), 119.3 (*C*-Ar), 122.0 (*C*-Ar), 122.3 (*C*-Ar), 127.4 (*C*-Ar), 128.1 (2 *C*-Ar), 128.5 (3 *C*-Ar), 136.4 (*C*-Ar), 136.7 (*C*-Ar), 156.5 (O*C*ONH), 170.4 (*C*ONH). IR (film) υ: 3312, 1704, 1652. ESI-HRMS: m/z calcd for $C_{33}H_{44}N_4O_6Na$ $[M+Na]^+$ 615.3153; found 615.3137.

3,6-Anhydro-2,7-dideoxy-*N*-[2-(1*H*-indol-3-yl)ethyl]-4,5-*O*-(1-methylethylidene)-7-[[*N*-[bis[(phenylmethoxy)carbonyl]]aminoiminomethyl]-*N*-[3-[bis[(phenylmethoxy)carbonyl]]guanidinopropyl]amino]-L-*galacto*-heptonamide 12

To a solution of **9** (282 mg, 0.5 mmol) in MeOH (10 mL) was added Pd/C (10%) (30 mg, 10% w/w). After stirring under H_2 atmosphere (30 psi) until completion of the reaction, the mixture was filtered through a pad of celite and the filtrate was concentrated *in vacuo*. The crude was solubilized in DMF (3 mL) and *N,N'*-bis(dibenzyloxycarbonyl)-*S*-methylisothiourea (1.0 mmol, 2eq.), $HgCl_2$ (134 mg, 0.5 mmol, 1 eq.) and Et_3N (153 mg, 1.5 mmol, 3 eq.) were added under argon atmosphere. After stirring 14 h at room temperature, the solvent was removed *in vacuo* and the residue was dissolved in EtOAc (40 mL). The organic layer was washed with water (5 mL), brine (5 mL), dried over $MgSO_4$, filtered and the solvent was removed under reduced pressure. The compound was purified by column chromatography (silica gel, cyH/EA) to provide **12** (131 mg, 25%).White solid. *Rf;* 0.52 (EA). $[\alpha]_D$= +5.1 (c = 0.2, $CHCl_3$). Mp = 65°C. ^{1}H NMR (250 MHz, MeOH d_4): δ = 1.21 (s, 3H, C*H*$_3$), 1.31 (s, 3H, C*H*$_3$), 1.80-1.92 (m, 2H, *H*-9), 2.46-2.49 (m, 2H, *H*-2), 2.91 (t, 2H, $J_{\alpha,\beta}$ = 7.0 Hz, *H*-β), 3.36-3.68 (m, 8H, *H*-7, *H*-8, *H*-10, *H*-α), 3.76-3.84 (m, 2H, *H*-3, *H*-6), 4.55 (dd, 1H, $J_{4,5}$ = 6.0, $J_{3,4}$ = 3.5 Hz, *H*-4), 4.62 (dd, 1H, $J_{5,6}$ = 4.0 Hz, *H*-5), 4.96-5.03 (m, 4H, C*H*$_2$-Ph), 5.07 (s, 2H, C*H*$_2$-Ph), 5.12 (s, 2H, C*H*$_2$-Ph), 6.94-7.09 (m, 3H, *H*-Ar), 7.22-7.37 (m, 21H, *H*-Ar), 7.55 (d, 1H, *J* = 8.0 Hz, *H*-Ar). ^{13}C NMR (62.9 MHz, MeOH d_4): δ = 25.0 (*C*H$_3$), 26.1 (*C*-β), 26.3 (*C*H$_3$), 27.8 (*C*-9), 36.7 (*C*-2), 39.4 (*C*-10), 41.3 (*C*-α), 41.5 (*C*-8), 48.9 (*C*-7), 68.4 (*C*H$_2$-Ph), 68.7 (2 *C*H$_2$-Ph), 69.2 (*C*H$_2$-Ph), 79.7 (*C*-3), 80.1 (*C*-6), 82.1 (*C*-4), 83.0 (*C*-5), 112.3 (*C*-Ar), 113.3 (*C*-Ar), 113.7 (*C*(CH$_3$)$_2$), 119.4 (*C*-Ar), 119.7 (*C*-Ar), 122.4 (*C*-Ar), 123.5 (*C*-Ar), 128.8 (*C*-Ar), 128.9

(*C*-Ar), 129.0 (2 *C*-Ar), 129.1 *(C*-Ar), 129.2 (2 *C*-Ar), 129.3 (*C*-Ar), 129.5 (8 *C*-Ar), 129.6 (*C*-Ar), 129.7 (4 *C*-Ar), 136.5 (2 *C*-Ar), 138.1 (*C*-Ar), 138.2 (2 *C*-Ar), 154.6 (2 *C*=N), 157.4 (2 NH*C*OO), 164.8 (2 NH*C*OO), 172.7 (*C*ONH). IR (film) υ: 1754, 1730, 1640. ESI-HRMS: m/z calcd for $C_{57}H_{62}N_8O_{12}Na$ $[M+Na]^+$ 1073.4379; found 1073.4374.

3,6-Anhydro-2,7-dideoxy-*N*-[2-(1*H*-indol-3-yl)ethyl]-4,5-*O*-(1-methylethylidene)-7-[[*N*-[bis[(phenylmethoxy)carbonyl]]aminoiminomethyl]-*N*-[4-[bis[(phenylmethoxy)carbonyl]]guanidinobutyl]amino]-L-*galacto*-heptonamide 13

Prepared starting from **10** (289 mg, 0.5 mmol) following procedure described for **12**. Yield: 25% (133 mg). White solid. *Rf;* 0.59 (EA). $[\alpha]_D$= +10.3 (c = 0.2, $CHCl_3$). Mp = 68°C. ^{1}H NMR (250 MHz, MeOH d_4): δ = 1.25 (s, 3H, C*H*$_3$), 1.35 (s, 3H, C*H*$_3$), 1.42-1.69 (m, 4H, *H*-9, *H*-10), 2.50-2.59 (m, 2H, *H*-2), 2.94 (t, 2H, $J_{\alpha,\beta}$ = 7.0 Hz, *H*-β), 3.37-3.54 (m, 6H, *H*-8, *H*-11, *H*-α), 3.61-3.71 (m, 2H, *H*-7), 3.76-3.84 (m, 2H, *H*-3, *H*-6), 4.59-4.68 (m, 2H, *H*-4, *H*-5), 5.04-5.06 (m, 4H, C*H*$_2$-Ph), 5.11 (s, 2H, C*H*$_2$-Ph), 5.20 (s, 2H, C*H*$_2$-Ph), 6.98-7.12 (m, 3H, *H*-Ar), 7.25-7.43 (m, 21H, *H*-Ar), 7.58 (d, 1H, *J* = 8.0 Hz, *H*-Ar). ^{13}C NMR (62.9 MHz, MeOH d_4): δ = 25.1 (*C*H$_3$), 26.3 (*C*H$_3$), 26.4, 27.2, 28.2 (*C*-β, *C*-9, *C*-10), 36.9 (*C*-2), 41.5 (*C*-α), 41.7 (*C*-8), 50.0 (*C*-7, *C*-12), 68.5 (*C*H$_2$-Ph), 68.9 (2 *C*H$_2$-Ph), 69.4 (*C*H$_2$-Ph), 79.8 (*C*-3), 80.4 (*C*-6), 82.3 (*C*-4), 83.1 (*C*-5), 112.4 (*C*-Ar), 113.4 (*C*-Ar), 113.9 (*C*(CH$_3$)$_2$), 119.5 (*C*-Ar), 119.8 (*C*-Ar), 122.5 (*C*-Ar), 123.6 (*C*-Ar), 128.9 (*C*-Ar), 128.9 (2 *C*-Ar), 129.0 (2 *C*-Ar), 129.1 (*C*-Ar), 129.2 (2 *C*-Ar), 129.4 (*C*-Ar), 129.6 (8 *C*-Ar), 129.7 (*C*-Ar), 129.9 (4 *C*-Ar), 136.7 (2 *C*-Ar), 138.3 (2 *C*-Ar), 138.4 (*C*-Ar), 154.8 (*C*=N), 154.9 (*C*=N), 157.5 (2 NH*C*OO), 165.0 (2 NH*C*OO), 172.9 (*C*ONH). IR (film) υ: 1728, 1638, 1622. ESI-HRMS: m/z calcd for $C_{58}H_{64}N_8O_{12}Na$ $[M+Na]^+$ 1087.4536; found 1087.4492.

3,6-Anhydro-2,7-dideoxy-*N*-[2-(1*H*-indol-3-yl)ethyl]-4,5-*O*-(1-methylethylidene)-7-[[*N*-[bis[(phenylmethoxy)carbonyl]]aminoiminomethyl]-*N*-[5-[bis[(phenylmethoxy)carbonyl]]guanidinopentyl]amino]- L-*galacto*-heptonamide 14

Prepared starting from **11** (296 mg, 0.5 mmol) following procedure described for **12**. Yield: 35% (188 mg). White solid. *Rf;* 0.63 (EA). $[\alpha]_D$= +12.3° (c = 0.5, $CHCl_3$). Mp = 70°C. ^{1}H NMR (400 MHz, MeOH d_4): δ = 1.24 (s, 3H, C*H*$_3$), 1.26-1.33 (m, 2H, *H*-10), 1.35 (s, 3H, C*H*$_3$), 1.50-1.64 (m, 4H, *H*-9, *H*-11), 2.50-2.52 (m, 2H, *H*-2), 2.93 (t, 2H, $J_{\alpha,\beta}$ = 7.5 Hz, *H*-β), 3.30-3.51 (m, 6H, *H*-8, *H*-12, *H*-α), 3.58-3.68 (m, 2H, *H*-7), 3.74 (m, 1H, *H*-6), 3.84 (m, 1H, *H*-3), 4.59-4.64 (m, 2H, *H*-4, *H*-5), 5.03 (s, 4H, C*H*$_2$-Ph), 5.10 (s, 2H, C*H*$_2$-Ph), 5.19 (s, 2H, C*H*$_2$-Ph), 6.98-7.09 (m, 3H, *H*-Ar), 7.25-7.40 (m, 21H, *H*-Ar), 7.57 (d, 1H, *J* = 8.0 Hz, *H*-Ar). ^{13}C NMR (100 MHz, MeOH d_4): δ = 24.7 (*C*-10), 24.9 (*C*H$_3$), 26.1 (*C*-β), 26.3 (CH$_3$) 27.6, 29.4, 30.7 (*C*-9, *C*-11), 36.7 (*C*-2), 41.4 (*C*-α), 41.8 (*C*-8), 49.1 (*C*-7), 50.1 (*C*-12), 68.4 (*C*H$_2$-Ph), 68.7 (2 *C*H$_2$-Ph), 69.3 (*C*H$_2$-Ph), 79.6 (*C*-3), 80.2 (*C*-6), 82.1 (*C*-4), 83.0 (*C*-5), 112.3 (*C*-Ar), 113.3 (*C*-Ar), 113.7 (*C*(CH$_3$)$_2$), 119.4 (*C*-Ar), 119.6 (*C*-Ar), 122.3 (*C*-Ar), 123.5 (*C*-Ar), 128.9 (*C*-Ar), 129.0 (4 *C*-Ar), 129.1 (4 *C*-Ar), 129.5 (8 *C*-Ar), 129.7 (4 *C*-Ar), 136.5 (2 *C*-Ar), 138.1 (*C*-Ar), 138.3 (2 *C*-Ar), 154.8 (2 *C*=N), 157.3 (2 NH*C*OO), 164.8 (2 NH*C*OO), 172.8 (*C*ONH). IR (film) υ: 1759, 1728, 1643. ESI-HRMS: m/z calcd for $C_{59}H_{66}N_8O_{12}Na$ $[M+Na]^+$ 1101.4692; found 1101.4706.

3,6-Anhydro-2,7-dideoxy-*N*-[2-(1*H*-indol-3-yl)ethyl]-4,5-*O*-(1-methylethylidene)-7-[*N*-(aminoiminomethyl)-*N*-(3-guanidinopropyl)amino]-L-*galacto*-heptonamide 15

To a solution of **12** (110 mg, 0.1 mmol) in MeOH (10 mL) was added Pd/C (10%) (10% w/w). After stirring under H_2 atmosphere (30 psi) until completion of the reaction, the mixture was filtered through a pad of celite and the filtrate was concentrated *in vacuo*. The crude was purified by column chromatography (C-18 silica gel, H_2O/CAN/0.1%TFA) and lyophilized to provide **15** (46 mg, 90%). White solid. *Rf;* 0.27 (H_2O/MeCN/TFA: 6/4/0.1%). HPLC (60/40): t_R = 4.920 min. $[\alpha]_D$= +11.2° (c = 0.1, MeOH). ^{1}H NMR (250 MHz, MeOH d_4): δ = 1.30 (s,

3H, C*H*$_3$), 1.45 (s, 3H, C*H*$_3$), 1.88-1.94 (m, 2H, *H*-9), 2.58 (d, 2H, J_{gem} = 7.0 Hz, *H*-2), 2.95 (t, 2H, $J_{\alpha,\beta}$ = 7.0 Hz, *H*-β), 3.18 (t, 2H, $J_{9,10}$ = 7.0 Hz, *H*-10), 3.40-3.78 (m, 7H, *H*-6, *H*-7, *H*-8, *H*-α), 3.91 (m,1H, *H*-3), 4.67 (dd, 1H, $J_{4,5}$= 6.0, $J_{3,4}$ = 3.5 Hz, *H*-4), 4.77 (m, 1H, *H*-5), 6.96-7.11 (m, 3H, *H*-Ar), 7.33 (d, 1H, *J* = 8.0 Hz, *H*-Ar), 7.56 (d, 1H, *J* = 8.0 Hz, *H*-Ar). ^{13}C NMR (62.9 MHz, MeOH d$_4$): δ = 23.5 (*C*H$_3$), 25.0, 25.1 (*C*-β, *C*H$_3$), 25.8 (*C*-9), 35.3 (*C*-2), 38.6 (*C*-7), 40.4 (*C*-α), 45.0 (*C*-8), 48.7 (*C*-10), 78.8 (*C*-3), 79.0 (*C*-6), 81.1 (*C*-4), 81.6 (*C*-5), 111.1 (*C*-Ar), 112.1 (*C*-Ar), 112.6 (*C*(CH$_3$)$_2$), 118.1 (*C*-Ar), 118.4 (*C*-Ar), 121.2 (*C*-Ar), 122.3 (*C*-Ar), 127.7 (*C*-Ar), 137.0 (*C*-Ar), 157.6 (*C*=N), 157.7 (*C*=N), 171.7 (*C*ONH). IR (film) υ: 3351, 1651, 1607. ESI-HRMS: m/z calcd for $C_{25}H_{38}N_8O_4Na$ $[M+Na]^+$ 537.2908; found 537.2930.

3,6-Anhydro-2,7-dideoxy-*N*-[2-(1*H*-indol-3-yl)ethyl]-4,5-*O*-(1-methylethylidene)-7-[*N*-(aminoiminomethyl)-*N*-(4-guanidinobutyl)amino]-L-*galacto*-heptonamide 16

Prepared starting from **13** (110 mg, 0.1 mmol) following procedure described for the synthesis of **15**. Yield: 85% (45 mg). White solid. *Rf;* 0.23 (H$_2$O/MeCN/TFA: 6/4/0.1%). HPLC (60/40): t_R = 5.138 min. $[\alpha]_D$= +5.0° (c = 0.1, MeOH). ^{1}H NMR (250 MHz, MeOH d$_4$): δ = 1.30 (s, 3H, C*H*$_3$), 1.46 (s, 3H, C*H*$_3$), 1.59-1.71 (m, 4H, *H*-9, *H*-10), 2.57-2.61 (m, 2H, *H*-2), 2.95 (t, 2H, $J_{\alpha,\beta}$ = 7.0 Hz, *H*-β), 3.20 (t, 2H, *H*-11), 3.38-3.69 (m, 6H, *H*-7, *H*-8, *H*-α), 3.78 (m, 1H, *H*-6), 3.94 (m, 1H, *H*-3), 4.68 (dd, 1H, $J_{4,5}$ = 6.0, $J_{3,4}$ = 3.0 Hz, *H*-4), 4.83 (m, 1H, *H*-5), 6.97-7.11 (m, 3H, *H*-Ar), 7.34 (d, 1H, *J* = 8.0 Hz, *H*-Ar), 7.56 (d, 1H, *J* = 8.0 Hz, *H*-Ar). ^{13}C NMR (62.9 MHz, MeOH d$_4$): δ = 24.7 (*C*H$_3$), 25.3 (*C*H$_3$), 26.2, 26.3, 26.8 (*C*-β, *C*-9, *C*-10), 36.4 (*C*-2), 41.6 (*C*-α), 42.2 (*C*-7), 49.9 (*C*-8), 50.4 (*C*-11), 79.9 (*C*-3), 80.2 (*C*-6), 82.3 (*C*-4), 82.8 (*C*-5), 112.3 (*C*-Ar), 113.2 (*C*-Ar), 113.7 (*C*(CH$_3$)$_2$), 119.3 (*C*-Ar), 119.6 (*C*-Ar), 122.3 (*C*-Ar), 123.5 (*C*-Ar), 128.8 (*C*-Ar), 138.1 (*C*-Ar), 158.6 (*C*=N), 158.8 (*C*=N), 172.9 (*C*ONH). IR (film) υ: 3379, 1681. ESI-HRMS: m/z calcd for $C_{26}H_{40}N_8O_4Na$ $[M+Na]^+$ 551.3065; found 551.3039.

3,6-Anhydro-2,7-dideoxy-*N*-[2-(1*H*-indol-3-yl)ethyl]-4,5-*O*-(1-methylethylidene)-7-[*N*-(aminoiminomethyl)-*N*-(5-guanidinopentyl)amino]-L-*galacto*-heptonamide 17

Prepared starting from **14** (160 mg, 0.15 mmol) following procedure described for the synthesis of **15**. Yield: 94% (76 mg). White solid. *Rf;* 0.20 (H$_2$O/MeCN/TFA: 6/4/0.1%). HPLC (60/40): t_R = 5.404 min. $[\alpha]_D$= +6.9° (c = 0.2, MeOH). ^{1}H NMR (250 MHz, MeOH d$_4$): δ = 1.30 (s, 3H, C*H*$_3$), 1.46 (s, 3H, C*H*$_3$), 1.30-1.42 (m, 2H, *H*-10), 1.56-1.70 (m, 4H, *H*-9, *H*-11), 2.59 (d, 2H, $J_{2,3}$ = 6.5 Hz, *H*-2), 2.94 (t, 2H, $J_{\alpha,\beta}$ = 7.5 Hz, *H*-β), 3.17 (t, 2H, $J_{11,12}$ = 7.0 Hz, *H*-12), 3.36-3.67 (m, 6H, *H*-α, *H*-8, *H*-7), 3.76 (m, 1H, *H*-6), 3.94 (m, 1H, *H*-3), 4.68 (dd, 1H, $J_{4,5}$ = 6.0, $J_{5,6}$ = 3.5 Hz, *H*-5), 4.77 (dd, 1H, $J_{3,4}$ = 3.5 Hz, *H*-4), 6.97-7.12 (m, 3H, *H*-Ar), 7.33 (d, 1H, *J* = 8.0 Hz, *H*-Ar), 7.57 (d, 1H, *J* = 8.0 Hz, *H*-Ar). ^{13}C NMR (62.9 MHz, MeOH d$_4$): δ = 24.6 (*C*H$_3$), 24.7 (*C*H$_3$), 26.2, 26.3 (*C*-β, *C*-10), 27.6, 29.6 (*C*-9, *C*-11), 36.5 (*C*-2), 41.6 (*C*-7), 42.3 (*C*-α), 49.7 (*C*-8), 50.7 (*C*-12), 79.9 (*C*-3), 80.2 (*C*-6), 82.3 (*C*-4), 82.8 (*C*-5), 112.3 (*C*-Ar), 113.3 (*C*(CH$_3$)$_2$), 113.7 (*C*-Ar), 119.3 (*C*-Ar), 119.6 (*C*-Ar), 122.3 (*C*-Ar), 123.4 (*C*-Ar), 128.8 (*C*-Ar), 138.2 (*C*-Ar), 158.7 (*C*=N), 158.8 (*C*=N), 172.8 (*C*ONH). IR (film) υ: 3327, 1657, 1617. ESI-HRMS: m/z calcd for $C_{27}H_{42}N_8O_4Na$ $[M+Na]^+$ 565.3221; found 565.3200.

3,6-Anhydro-2-deoxy-4,5-*O*-(1-methylethylidene)-7-*O*-acetyl-D-*altro*-heptoanic acid methyl ester 18

To a stirred solution of **3** (2.5 g, 8.7 mmol) in EtOAc (20 mL) was added Pd/C (10%) (1 g, 40% w/w). The mixture was stirred under H$_2$ atmosphere (30 psi) for 18h. The reaction mixture was then filtered through a pad of celite and the solvent was removed *in vacuo*. The compound was purified by column chromatography (silica gel, cyH/EA) to provide **18** (2.1 g, 85%). Colorless gum. *Rf;* 0.38 (cH/EA: 2/1). $[\alpha]_D$= -13.3° (c = 0.27, CHCl$_3$). ^{1}H NMR (250 MHz, CDCl$_3$): δ = 1.34 (s, 3H, C*H*$_3$), 1.49 (s, 3H, C*H*$_3$), 2.09 (s, 3H, OC*H*$_3$), 2.72 (dd, 1H, J_{gem} = 16.0, $J_{2,3}$ = 5.0 Hz, *H*-2), 2.81 (dd, 1H, $J_{2',3}$ = 6.0 Hz, *H*-2'), 3.71 (s, 3H, COOC*H*$_3$), 4.05 (dd, 1H,

J_{gem} = 11.0, $J_{6,7}$ = 4.5 Hz, *H*-7), 4.13-4.28 (m, 2H, *H*-7', *H*-6), 4.36 (m, 1H, *H*-3), 4.67 (d, 1H, $J_{4,5}$ = 6.0 Hz, *H*-5), 4.80 (dd, 1H, *H*-4). ^{13}C NMR (62.9 MHz, $CDCl_3$): δ = 20.7 (*C*H_3COO), 24.8, 26.0 (C(*C*H_3)$_2$), 34.0 (*C*-2), 51.5 (*C*H_3OCO), 63.3 (*C*-7), 77.3, 81.1, 81.4, 82.7 (*C*-3, *C*-4, *C*-5, *C*-6), 112.6 (*C*(CH$_3$)$_2$), 170.3, 171.2 (2 *C*=O). IR (film) υ: 2990, 2943, 1742. ESI-HRMS: m/z calcd for $C_{13}H_{20}O_7Na$ $[M+Na]^+$ 311.1101; found 311.1119.

3,6-Anhydro-2-deoxy-4,5-*O*-(1-methylethylidene)-D-*altro*-heptonic acid methyl ester 19

To a stirred solution of **18** (2.0 g, 6.9 mmol) in methanol (50 mL) was added a catalytic amount of sodium at 0°C under argon atmosphere. After completion of the reaction (approx. 1h), acidic resin Amberlite IR-120 was added until pH = 4. After filtration, the solvent was removed under reduced pressure to provide **19** (1.65 g, 97%). Colorless gum. *Rf;* 0.10 (cH/EA: 2/1). $[\alpha]_D$= +5.4° (c = 0.52, $CHCl_3$). ^{1}H NMR (250 MHz, $CDCl_3$): δ = 1.33 (s, 3H, C(C*H*$_3$)$_2$), 1.49 (s, 3H, C(C*H*$_3$)$_2$), 1.91 (br s, 1H, O*H*), 2.72 (dd, 1H, J_{gem} = 16.5, $J_{2,3}$ = 7.0 Hz, *H*-2), 2.80 (dd, 1H, $J_{2',3}$ = 7.0, *H*-2'), 3.60-3.66 (m, 2H, *H*-7, *H*-7'), 3.71 (s, 3H, OC*H*$_3$), 4.14 (td, 1H, $J_{6,7}$ = 7.0, $J_{5,6}$ = 1.0 Hz, *H*-6), 4.39 (td, 1H, $J_{3,4}$ = 4.0 Hz, *H*-3), 4.66 (dd, 1H, $J_{4,5}$ = 6.0 Hz, *H*-5), 4.78 (dd, 1H, *H*-4). ^{13}C NMR (62.9 MHz, $CDCl_3$): δ = 25.2 (*C*H$_3$), 26.4 (*C*H$_3$), 34.5 (*C*-2), 51.9 (O*C*H$_3$), 62.1 (*C*-7), 77.2 (*C*-3), 81.5, 82.6, 84.3 (*C*-4, *C*-5, *C*-6), 113.0 (*C*(CH$_3$)$_2$), 171.8 (*C*=O). IR (film) υ: 3464, 2990, 2943, 1737. ESI-HRMS: m/z calcd for $C_{11}H_{18}O_6Na$ $[M+Na]^+$ 269.0996; found 269.1005.

3,6-Anhydro-2-deoxy-7-*O*-[(*tert*-butyldimethyl)silyl]-4,5-*O*-(1-methylethylidene)-D-*altro*-heptonic acid methyl ester 20

To a solution of **19** (1.5 g, 6.1 mmol, 1 eq.) in DMF (12 mL), were added *tert*-butyldimethylsilyl chloride (1.1g, 6.71 mmol, 1.1 eq.) and imidazole (1.04 g, 15.25 mmol, 2.5 eq.) and the mixture was stirred at room temperature until completion of the reaction. The reaction was quenched by addition of EtOH and the solvent were removed *in vacuo*. The residue was diluted in EtOAc (40 mL) and washed with water (20 mL) and brine (20 mL). The organic layer was dried over $MgSO_4$, filtered and concentrated *in vacuo*. The compound was purified by column chromatography (silica gel, cyH/EA) to provide **20** in quantitative yield (2.2 g). Colorless gum. *Rf;* 0.76 (cH/EA: 2/1). $[\alpha]_D$= -12.7° (c = 0.45, $CHCl_3$). ^{1}H NMR (250 MHz, $CDCl_3$): δ = 0.06 (s, 6H, C*H*$_3$), 0.89 (s, 9H, C(C*H*$_3$)$_3$), 1.34 (s, 3H, C*H*$_3$), 1.48 (s, 3H, C*H*$_3$), 2.67 (dd, 1H, J_{gem} = 16.0, $J_{2,3}$ = 4.5 Hz, *H*-2), 2.75 (dd, 1H, $J_{2',3}$ = 4.0, *H*-2'), 3.69 (s, 3H, COOC*H*$_3$), 3.64-3.75 (m, 2H, *H*-7, *H*-7'), 4.07 (t, 1H, $J_{6,7}$ = 3.5 Hz, *H*-6), 4.50 (m, 1H, *H*-3), 4.75 (m, 1H, *H*-5), 4.82 (d, 1H, $J_{4,5}$ = 6.0 Hz, *H*-4). ^{13}C NMR (62.9 MHz, $CDCl_3$): δ = -5.5 (Si(*C*H$_3$)$_2$), 18.2 (*C*(CH$_3$)$_3$), 25.1 (C(*C*H$_3$)$_2$), 25.9 (C(*C*H$_3$)$_3$), 26.3 (C(*C*H$_3$)$_2$), 34.9 (*C*-2), 51.7 (*C*H$_3$OCO), 64.9 (*C*-7), 78.6, 81.9, 83.3, 84.3 (*C*-3, *C*-4, *C*-5, *C*-6), 112.3 (*C*(CH$_3$)$_2$), 171.8 (*C*=O). IR (film) υ: 2976, 2957, 2933, 2853, 1742. ESI-HRMS: m/z calcd for $C_{17}H_{32}O_6SiNa$ $[M+Na]^+$ 383.1860; found 383.1864.

3,6-Anhydro-7-*O*-[(*tert*-butyldimethyl)silyl]-2-deoxy-4,5-*O*-(1-methylethylidene)-D-*altro*-heptonic acid 21

To a solution of **20** (500 mg, 1.39 mmol) in THF (15 mL) and water (5 mL) was added LiOH (100 mg, 4.17 mmol, 3 eq.) and the mixture was stirred until completion of the reaction monitored by tlc. THF was removed under reduced pressure, CH_2Cl_2 (20 mL) was added and the pH was adjusted to 2-3 with 1N HCl. The organic layer was washed with brine and dried over $MgSO_4$, filtered and concentrated to provide **21** (312 mg, 65%). Colorless gum. *Rf;* 0.56 (cH/EA: 2/1). $[\alpha]_D$= -21.1° (c = 0.1, $CHCl_3$). ^{1}H NMR (250 MHz, $CDCl_3$): δ = 0.06 (s, 6H, C*H*$_3$), 0.89 (s, 9H, C(C*H*$_3$)$_3$), 1.35 (s, 3H, C*H*$_3$), 1.50 (s, 3H, C*H*$_3$), 2.71 (dd, 1H, J_{gem} = 16.0, $J_{2,3}$ = 4.5 Hz, *H*-2), 2.80 (dd, 1H, $J_{2',3}$ = 4.0, *H*-2'), 3.69 (dd, 1H, J_{gem} = 11.0, $J_{6,7}$ = 3.5 Hz, *H*-

7), 3.76 (dd, 1H, $J_{6,7'}$ = 3.5 Hz, *H*-7'), 4.13 (t, 1H, *H*-6), 4.51 (m, 1H, *H*-3), 4.76 (m, 1H, *H*-5), 4.84 (d, 1H, $J_{4,5}$ = 6.0 Hz, *H*-4). ^{13}C NMR (62.9 MHz, $CDCl_3$): δ = -5.5 (Si*C*H$_3$), -5.4 (Si*C*H$_3$), 18.3 (*C*(CH$_3$)$_3$), 25.0 (C(*C*H$_3$)$_2$), 26.0 (C(*C*H$_3$)$_3$), 26.2 (C(*C*H$_3$)$_2$), 34.9 (*C*-2), 65.0 (*C*-7), 78.5, 81.9, 83.3, 84.4 (*C*-3, *C*-4, *C*-5, *C*-6), 112.6 (*C*(CH$_3$)$_2$), 176.3 (*C*=O). IR (film) υ: 2933, 2853, 2251, 1714. ESI-HRMS: m/z calcd for $C_{16}H_{30}O_6SiNa$ $[M+Na]^+$ 369.1704; found 369.1701.

3,6-anhydro-7-*O*-[(*tert*-butyldimethyl)silyl]-2-deoxy-*N*-[2-(1*H*-indol-3-yl)ethyl]-4,5-*O*-(1-methylethylidene)-D-*altro*-heptonamide 22

To a stirred solution of **21** (260 mg, 0.75 mmol, 1 eq.) in CH_2Cl_2 (10 mL) were added tryptamine (132 mg, 0.83 mmol, 1.1 eq.) and EDC (159 mg, 0.83 mmol, 1.1 eq.) at 0°C under argon atmosphere. After stirring overnight at room temperature, the solvent was evaporated under reduced pressure. The residue was diluted with CH_2Cl_2 (20 mL) and the organic layer was washed with a saturated aqueous solution of $NaHCO_3$ (10 mL), dried over $MgSO_4$, filtered and the solvent was removed *in vacuo*. The product was purified by column chromatography (silica gel, cyH/EA) to provide **22** (292 mg, 80%). Colorless gum. *Rf;* 0.75 (CH_2Cl_2/MeOH 9/1). $[\alpha]_D$= -0.1 (c = 0.34, $CHCl_3$). ^{1}H NMR (250 MHz, $CDCl_3$): δ = 0.04 (s, 6H, C*H*$_3$), 0.88 (s, 9H, C(C*H*$_3$)$_3$), 1.30 (s, 3H, C*H*$_3$), 1.45 (s, 3H, C*H*$_3$), 2.53 (d, 2H, $J_{2,3}$ = 6.5 Hz, *H*-2, *H*-2'), 2.98 (t, 2H, *J* = 7.0 Hz, *H*-β), 3.53-3.69 (m, 4H, *H*-7, *H*-7', *H*-α), 4.03 (t, 1H, $J_{6,7}$ = 4.0 Hz, *H*-6), 4.38 (m, 1H, *H*-3), 4.63 (m, 1H, *H*-5), 4.77 (d, 1H, $J_{4,5}$ = 6.0 Hz, *H*-4), 6.24 (t, 1H, *J* = 5.0 Hz, CON*H*), 7.06-7.24 (m, 3H, *H*-Ar), 7.37 (d, 1H, *H*-Ar), 7.62 (d, 1H, *H*-Ar), 8.03 (br s, 1H, N*H*). ^{13}C NMR (62.9 MHz, $CDCl_3$): *δ* = -5.5 (Si*C*H$_3$), -5.4 (Si*C*H$_3$), 18.3 (*C*(CH$_3$)$_3$), 24.9 (C(*C*H$_3$)$_2$), 25.4 (*C*-β), 26.0 (C(*C*H$_3$)$_3$), 26.3 (C(*C*H$_3$)$_2$), 37.6 (*C*-2), 39.9 (*C*-α), 64.4 (*C*-7), 79.0, 82.1, 83.1, 84.5 (*C*-3, *C*-4, *C*-5, *C*-6), 111.3 (*C*(CH$_3$)$_2$), 112.3, 113.2, 118.9, 119.5, 122.1, 122.2, 127.5, 136.5 (8 *C*-Ar), 170.9(*C*=O). IR (film) υ: 3327, 2947, 2933, 2862, 1650. ESI-HRMS: m/z calcd for $C_{26}H_{40}N_2O_5SiNa$ $[M+Na]^+$ 551.2599; found 551.2549.

3,6-Anhydro-2-deoxy-*N*-[2-(1*H*-indol-3-yl)ethyl]-4,5-*O*-(1-methylethylidene)-D-*altro*-heptonamide 23

To a solution of **22** (248 mg, 0.51 mmol, 1 eq.) in dry THF (3 mL) was added dropwise TBAF (1M in THF) (0.77 mL, 0.77 mmol, 1.5 eq.) at 0°C and the reaction mixture was stirred overnight under argon atmosphere. Water (5 mL) was added and the aqueous layer was extracted with EtOAc (2x15 mL). The combined organic layers were washed with brine (10 mL), dried over $MgSO_4$, filtered and the solvent was removed *in vacuo*. The product was purified by flash chromatography (silica gel, cyH/EA) to provide **23** (181 mg, 95%). Colorless gum. *Rf;* 0.61 (CH_2Cl_2/MeOH 9/1). $[\alpha]_D$= -6.3 (c = 0.09, $CHCl_3$). ^{1}H NMR (250 MHz, $CDCl_3$): δ = 1.27 (s, 3H, C*H*$_3$), 1.44 (s, 3H, C*H*$_3$), 2.40 (br s, 1H, O*H*), 2.53 (d, 2H, $J_{2,3}$ = 6.5 Hz, *H*-2, *H*-2'), 2.97 (t, 2H, *J* = 6.5 Hz, *H*-β), 3.51 (d, 2H, $J_{6,7}$ = 5.5 Hz, *H*-7, *H*-7'), 3.56-3.66 (m, 2H, *H*-α), 4.06 (t, 1H, *H*-6), 4.30 (m, 1H, *H*-3), 4.55-4.63 (m, 2H, *H*-5, *H*-4), 6.19 (t, 1H, *J* = 5.0 Hz, CON*H*), 7.04-7.23 (m, 3H, *H*-Ar), 7.37 (d, 1H, *H*-Ar), 7.60 (d, 1H, *H*-Ar), 8.36 (br s, 1H, N*H*). ^{13}C NMR (62.9 MHz, $CDCl_3$): δ = 24.9 (C(*C*H$_3$)$_2$), 25.2 (*C*-β), 26.3 (C(*C*H$_3$)$_2$), 37.1 (*C*-2), 39.9 (*C*-α), 62.3 (*C*-7), 77.7, 81.8, 82.6, 84.7 (*C*-3, *C*-4, *C*-5, *C*-6), 111.4 (*C*(CH$_3$)$_2$), 112.6, 112.9, 118.8, 119.5, 122.2, 122.4, 127.5, 136.5 (8 *C*-Ar), 171.1(*C*=O). IR (film) υ: 3336, 2985, 2943, 2876, 1650. ESI-HRMS: m/z calcd for $C_{20}H_{26}N_2O_5Na$ $[M+Na]^+$ 397.1734; found 397.1731.

3,6-Anhydro-2-deoxy-*N*-[2-(1*H*-indol-3-yl)ethyl]-7-(4-methylbenzenesulfonate)-4,5-*O*-(1-methylethylidene)-D-*altro*-heptonamide 24

Prepared starting from **23** (160 mg, 0.43 mmol) following procedure described for **8**. Yield: 87% (197 mg). White solid. *Rf;* 0.67 (EA). $[\alpha]_D$= +10.7 (c = 1.0, $CHCl_3$). Mp = 64°C. ^{1}H NMR (250 MHz, $CDCl_3$): δ = 1.22 (s, 3H, C*H*$_3$), 1.42 (s, 3H, C*H*$_3$), 2.48 (s, 3H, Ph-C*H*$_3$), 2.46-2.52

(m, 2H, *H*-2), 3.02 (t, 2H, $J_{\alpha,\beta}$ = 6.5 Hz, *H*-β), 3.60-3.68 (m, 2H, *H*-α), 3.92 (dd, 1H, $J_{6,7}$ = 4.5, J_{gem} = 11.0 Hz, *H*-7), 4.03 (dd, 1H, $J_{6,7'}$ = 5.0 Hz, *H*-7'), 4.13-4.23 (m, 2H, *H*-6, *H*-3), 4.45 (dd, 1H, $J_{4,5}$ = 6.0, $J_{5,6}$ = 4.0 Hz, *H*-5), 4.60 (d, 1H, *H*-4), 6.11 (t, 1H, *J* = 5.5 Hz, CON*H*), 7.09-7.44 (m, 6H, *H*-Ar), 7.63 (d, 1H, *J* = 8.0 Hz, *H*-Ar), 7.81 (d, 2H, *J* = 8.5 Hz, *H*-Ar), 8.33 (s, 1H, N*H*-Ar). ^{13}C NMR (62.9 MHz, $CDCl_3$): δ = 21.7 (*C*H_3-Ph), 24.7 (*C*H_3), 25.0 (*C*H_3), 26.1 (*C*-β), 37.2 (*C*-2), 39.5 (*C*-α), 69.5 (*C*-7), 78.8 (*C*-3), 81.4 (*C*-6, *C*-4), 82.3 (*C*-5), 111.3 (*C*$(CH_3)_2$), 112.8 (*C*-Ar), 112.9 (*C*-Ar), 118.7 (*C*-Ar), 119.4 (*C*-Ar), 122.1 (*C*-Ar), 122.3 (*C*-Ar), 127.4 (*C*-Ar), 127.9 (2 *C*-Ar), 130.1 (2 *C*-Ar), 132.2 (*C*-Ar), 136.4 (*C*-Ar), 145.4 (*C*-Ar), 170.1 (*C*=O). IR (film) υ: 3407, 3308, 1652. ESI-HRMS: m/z calcd for $C_{27}H_{32}N_2O_7SNa$ $[M+Na]^+$ 551.1822; found 551.1853.

3,6-Anhydro-2,7-dideoxy-*N*-[2-(1*H*-indol-3-yl)ethyl]-4,5-*O*-(1-methylethylidene)-7-[[2-[[(phenylmethoxy)carbonyl]amino]ethyl]amino]-D-*altro*-heptonamide 25

Prepared starting from **24** (170 mg, 0.32 mmol) following procedure described for **9**. Yield: 82% (144 mg). Colorless gum. *Rf;* 0.35 (CH_2Cl_2/MeOH 9/1). $[\alpha]_D$= +3.5 (c = 0.5, $CHCl_3$). ^{1}H NMR (400 MHz, $CDCl_3$): δ = 1.28 (s, 3H, C(C*H*$_3$)$_2$), 1.45 (s, 3H, C(C*H*$_3$)$_2$), 2.07 (br s, 1H, N*H*), 2.47 (dd, 1H, J_{gem} = 12.5, $J_{6,7}$ = 5.0 Hz, *H*-7), 2.53-2.59 (m, 3H, 2 *H*-2, *H*-7), 2.71 (t, 2H, $J_{8,9}$ = 6.0 Hz, *H*-8), 2.97 (t, 2H, $J_{\alpha,\beta}$ = 6.5 Hz, *H*-β), 3.23-3.27 (m, 2H, *H*-9), 3.58-3.64 (m, 2H, *H*-α), 4.09 (m, 1H, *H*-6), 4.14 (m, 1H, *H*-3), 4.47 (d, 1H, $J_{4,5}$ = 6.0 Hz, *H*-5), 4.56 (dd, 1H, $J_{3,4}$ = 4.0 Hz, *H*-4), 5.12 (s, 2H, C*H*$_2$-Ph), 5.39 (t, 1H, *J* = 5.0 Hz, CON*H*), 6.12 (t, 1H, *J* = 5.0 Hz, CON*H*), 7.05 (d, 1H, *J* = 2.0 Hz, *H*-Ar), 7.13 (m, 1H, *H*-Ar), 7.21 (m, 1H, *H*-Ar), 7.32-7.39 (m, 6H, *H*-Ar), 7.61 (d, 1H, *J* = 8.0 Hz, *H*-Ar), 8.52 (br s, 1H, N*H*). ^{13}C NMR (100 MHz, $CDCl_3$): *δ* = 24.9 (C(*C*H_3)$_2$), 25.1 (*C*-β), 26.2 (C(*C*H_3)$_2$), 36.6 (*C*-2), 39.7 (*C*-α), 40.3 (*C*-9), 48.3 (*C*-7), 48.6 (*C*-8), 66.7 (*C*H_2-Ph), 76.5 (*C*-3), 81.3 (*C*-4), 82.9 (*C*-6), 83.7 (*C*-5), 111.3 (*C*-Ar), 112.6 (*C*$(CH_3)_2$), 112.9 (*C*-Ar), 118.7 (*C*-Ar), 119.4 (*C*-Ar), 122.1 (*C*-Ar), 122.2 (*C*-Ar), 127.4 (*C*-Ar), 128.1 (*C*-Ar), 128.2 (2 *C*-Ar), 128.5 (2 *C*-Ar), 136.4 (*C*-Ar), 136.6 (*C*-Ar), 156.7 (O*C*ONH), 170.5 (*C*ONH). IR (film) υ: 3327, 1709, 1650. ESI-HRMS: m/z calcd for $C_{30}H_{38}N_4O_6Na$ $[M+Na]^+$ 573.2684; found 573.2707.

3,6-Anhydro-2,7-dideoxy-*N*-[2-(1*H*-indol-3-yl)ethyl]-4,5-*O*-(1-methylethylidene)-7-[[*N*-[bis[(phenylmethoxy)carbonyl]]aminoiminomethyl]-*N*-[2-[bis[(phenylmethoxy)carbonyl]]guanidinoethyl]amino]-D-*altro*-heptonamide 26

Prepared starting from **25** (120 mg, 0.22 mmol) following procedure described for **12**. Yield: 30% (68 mg). White solid. *Rf;* 0.50 (EA). $[\alpha]_D$= +11.4 (c = 0.5, $CHCl_3$). Mp = 70°C. ^{1}H NMR (400 MHz, MeOH-d$_4$): δ = 1.23 (s, 3H, C(C*H*$_3$)$_2$), 1.39 (s, 3H, C(C*H*$_3$)$_2$), 2.47 (dd, 1H, J_{gem} = 15.5, $J_{2,3}$ = 5.5 Hz, *H*-2), 2.56 (dd, 1H, $J_{2',3}$ = 8.0 Hz, *H*-2'), 2.90 (t, 2H, $J_{\alpha,\beta}$ = 6.5 Hz, *H*-β), 3.44-3.72 (m, 8H, 2 *H*-α, 2 *H*-7, 2 *H*-8, 2 *H*-9), 4.25-4.29 (m, 2H, *H*-6, *H*-3), 4.43 (d, 1H, $J_{4,5}$ = 6.0 Hz, *H*-5), 4.51 (dd, 1H, $J_{3,4}$ = 3.5 Hz, *H*-4), 5.00-5.01 (m, 6H, C*H*$_2$-Ph), 5.08 (s, 2H, C*H*$_2$-Ph), 6.96-7.08 (m, 3H, *H*-Ar), 7.19-7.36 (m, 21H, *H*-Ar), 7.54 (d, 1H, *J* = 8.0 Hz, *H*-Ar). ^{13}C NMR (100 MHz, MeOH-d$_4$): *δ* = 25.1 (C(*C*H_3)$_2$), 26.1 (*C*-β), 26.6 (C(*C*H_3)$_2$), 36.9 (*C*-2), 39.7 (*C*-7), 41.3 (*C*-α), 48.8 (*C*-8), 68.3 (*C*H_2-Ph), 68.8 (*C*H_2-Ph), 69.2 (2 *C*H_2-Ph), 78.0 (*C*-3, *C*-6), 82.6 (*C*-4), 84.5 (*C*-5), 112.3 (*C*-Ar), 113.3 (*C*-Ar), 113.6 (*C*$(CH_3)_2$), 119.4 (*C*-Ar), 119.6 (*C*-Ar), 122.3 (*C*-Ar), 123.5 (*C*-Ar), 128.8 (*C*-Ar), 129.0 (2 *C*-Ar), 129.2 (8 *C*-Ar), 129.4 (4 *C*-Ar), 129.5 (4 *C*-Ar), 129.6 (4 *C*-Ar), 136.4 (*C*-Ar), 138.1 (2 *C*-Ar), 154.4 (2 *C*-gua), 157.7 (2 O*C*ONH), 164.7 (2 O*C*ONH), 172.8 (*C*ONH). IR (film) υ: 3327, 1742, 1636, 1619. ESI-HRMS: m/z calcd for $C_{56}H_{60}N_8O_{12}Na$ $[M+Na]^+$ 1059.4223; found 1059.4244.

3,6-anhydro-2,7-dideoxy-*N*-[2-(1*H*-indol-3-yl)ethyl]-4,5-*O*-(1-methylethylidene)-7-[*N*-(aminoiminomethyl)-*N*-(2-guanidinoethyl)amino]-D-*altro*-heptonamide 27

Prepared starting from **26** (50 mg, 0.05 mmol) following procedure described for the synthesis of **15**. Yield: 90% (20 mg). White solid. *Rf;* 0.29 (H_2O/MeCN/TFA: 6/4/0.1%). HPLC (60/40): t_R = 4.740 min. $[\alpha]_D$= +23.0 (c = 0.2, MeOH). ^{1}H NMR (250 MHz, MeOH-d_4): δ = 1.33 (s, 3H, C(C*H*$_3$)$_2$), 1.48 (s, 3H, C(C*H*$_3$)$_2$), 2.52-2.66 (m, 2H, *H*-2), 2.97 (t, 2H, $J_{\alpha,\beta}$ = 7.0 Hz, *H*-β), 3.38-3.62 (m, 8H, *H*-α, *H*-7, *H*-8, *H*-9), 4.24 (m, 1H, *H*-6), 4.39 (m, 1H, *H*-3), 4.60 (d, 1H, $J_{4,5}$ = 6.0 Hz, *H*-5), 4.72 (dd, 1H, $J_{3,4}$ = 4.0 Hz, *H*-4), 6.99-7.14 (m, 3H, *H*-Ar), 7.36 (d, 1H, *J* = 8.0 Hz, *H*-Ar), 7.59 (d, 1H, *J* = 8.0 Hz, *H*-Ar). ^{13}C NMR (62.9 MHz, MeOH-d_4): δ = 25.1 (C(*C*H$_3$)$_2$), 26.2 (*C*-β), 26.6 (C(*C*H$_3$)$_2$), 36.7 (*C*-2), 40.2 (*C*-α), 41.6 (*C*-7), 49.7, 49.8 (*C*-8, *C*-9), 78.0 (*C*-3), 82.7, 84.0, 84.5 (*C*-4, *C*-5, *C*-6), 112.3 (*C*-Ar), 113.3 (*C*(CH$_3$)$_2$), 113.9 (*C*-Ar), 119.3 (*C*-Ar), 119.6 (*C*-Ar), 122.3 (*C*-Ar), 123.5 (*C*-Ar), 128.8 (*C*-Ar), 138.1 (*C*-Ar), 159.2, 159.7 (2 *C*=N), 172.9 (*C*ONH). IR (film) υ: 3374, 3189, 1674, 1607. ESI-HRMS: m/z calcd for $C_{24}H_{36}N_8O_4Na$ $[M+Na]^+$ 523.2752; found 523.2762.

***N,N'*-[Bis(phenylmethoxy)carbonyl]-*N''*-(but-3-ynyl)-guanidine 28**

To a cooled solution of butyn-4-ol (151 µL, 2.0 mmol, 1 eq.), *N,N'*-bis(benzyloxycarbonyl)guanidine (650 mg, 2.0 mmol, 1 eq.) and PPh_3 (630 mg, 2.4 mmol, 1.2 eq.) in dry toluene (10 mL), was added dropwise over 5 minutes a solution of diethylazodicarboxylate (DEAD, 378 µL, 2.4 mmol, 1.2 eq.) in toluene (5 mL) under argon atmosphere. The mixture was stirred at room temperature for 4h and the solvent was removed *in vacuo.* The crude was purified by flash chromatography (silica gel, cyH/EA) to provide **28** (700 mg, 92%). Colorless oil. *Rf;* 0.68 (cH/EA: 8/2). ^{1}H NMR (250 MHz, $CDCl_3$): δ = 1.92 (t, 1H, *J* = 2.0 Hz, C≡C-*H*), 2.57 (td, 2H, *J* = 7.0 Hz, C*H*$_2$), 4.22 (t, 2H, C*H*$_2$), 5.18 (s, 2H, C*H*$_2$-Ph), 5.28 (s, 2H, C*H*$_2$-Ph), 7.32-7.45 (m, 10H, 10 *H*-Ar), 9.29 (br s, 1H, N*H*), 9.47 (br s, 1H, N*H*). ^{13}C NMR (62.9 MHz, $CDCl_3$): δ = 17.5 (*C*H$_2$), 41.8 (*C*H$_2$), 66.0 (C≡*C*-H), 67.9, 69.0 (2 *C*H$_2$-Ph), 79.7 (*C*≡C-H), 126.8 (*C*-Ar), 126.9 (2 *C*-Ar), 127.3 (4 *C*-Ar), 127.7 (3 *C*-Ar), 133.5 (*C*-Ar), 135.9 (*C*-Ar), 154.7 (*C*=N), 159.1 (*C*=O), 162.7 (*C*=O). IR (film) υ: 3388, 3289, 3033, 2947, 1718, 1645, 1610, 1508. ESI-HRMS: m/z calcd for $C_{21}H_{22}N_3O_4$ $[M+H]^+$ 380.1616; found 380.1594.

***N,N'*-[Bis(phenylmethoxy)carbonyl]-*N''*-(pent-4-ynyl)-guanidine 29**

Prepared starting from pentyn-5-ol (185 µL, 2.0 mmol) following procedure described for **28**. Yield: 89% (700 mg). Colorless oil. *Rf;* 0.75 (cH/EA: 8/2). ^{1}H NMR (250 MHz, $CDCl_3$): δ = 1.78-1.90 (m, 3H, C*H*$_2$, C≡C-*H*), 2.19 (td, 2H, *J* = 7.0, *J* = 7.0 Hz, C*H*$_2$), 4.10 (t, 2H, C*H*$_2$), 5.16 (s, 2H, C*H*$_2$-Ph), 5.25 (s, 2H, C*H*$_2$-Ph), 7.28-7.42 (m, 10H, 10 *H*-Ar), 9.28 (br s, 1H, N*H*), 9.44 (br s, 1H, N*H*). ^{13}C NMR (62.9 MHz, $CDCl_3$): δ = 14.9 (*C*H$_2$), 26.4 (*C*H$_2$), 43.0 (*C*H$_2$), 66.0 (C≡*C*-H), 67.5, 67.9 (2 *C*H$_2$-Ph), 82.4 (*C*≡C-H), 126.7 (*C*-Ar), 126.8 (2 *C*-Ar), 127.3 (4 *C*-Ar), 127.6 (2 *C*-Ar), 127.7 (*C*-Ar), 133.6 (*C*-Ar), 135.9 (*C*-Ar), 154.8 (*C*=N), 159.5 (*C*=O), 162.8 (*C*=O). IR (film) υ: 3393, 3289, 3033, 2952, 1716, 1645, 1610, 1511. ESI-HRMS: m/z calcd for $C_{22}H_{24}N_3O_4$ $[M+H]^+$ 394.1777; found 394.1776.

3,6-Anhydro-7-azido-2,7-dideoxy-*N*-[2-(1*H*-indol-3-yl)ethyl]-4,5-*O*-(1-methylethylidene)-L-*galacto*-heptonamide 30

To a solution of **8** (1.0 g, 1.9 mmol) in DMF (10 mL) was added sodium azide (1.0 g, 15.2 mmol, 8 eq.) and the mixture was stirred for 2 days at 80°C. The solvent was removed in vacuo and the residue was diluted with EtOAc (50 mL) and washed with H_2O (20 mL) and brine (20 mL). The organic layer was dried over $MgSO_4$, filtered and the solvent was evaporated under reduced pressure. The compound was purified by flash chromatography (silica gel, cyH/EA) to provide **30** (667 mg, 88%). Colorless gum. *Rf;* 0.49 (CH_2Cl_2/MeOH: 9/1). $[\alpha]_D$= +9.7° (c = 0.4, $CHCl_3$). ^{1}H NMR (250 MHz, $CDCl_3$): δ = 1.27 (s, 3H, C(C*H*$_3$)), 1.42 (s, 3H, C(C*H*$_3$)), 2.57 (d, 2H, $J_{2,3}$ = 6.5 Hz, *H*-2), 2.99 (t, 2H, $J_{\alpha,\beta}$ = 6.5 Hz, *H*-β), 3.44 (dd, 2H, J_{gem} = 7.5, $J_{6,7}$ = 3.0 Hz,

H-7), 3.52-3.70 (m, 3H, *H*-6, *H*-α), 3.86 (td, 1H, $J_{3,4}$= 2.5 Hz, *H*-3), 4.59-4.66 (m, 2H, *H*-4, *H*-5), 6.04 (t, 1H, *J* = 5.0 Hz, CON*H*), 7.05-7.24 (m, 3H, *H*-Ar), 7.38 (d, 1H, *J* = 8.0, *H*-Ar), 7.62 (d, 1H, *J* = 8.0 Hz, *H*-Ar), 8.11 (s, 1H, N*H*-Ar). ^{13}C NMR (62.9 MHz, $CDCl_3$): δ = 24.7 (*C*H$_3$), 25.2 (*C*H$_3$), 25.9 (*C*-β), 36.3 (*C*-2), 40.0 (*C*-α), 49.6 (*C*-7), 78.4 (*C*-3), 80.0 (*C*-6), 80.7 (*C*-4), 81.5 (*C*-5), 111.4 (*C*(CH$_3$)$_2$), 112.8 (*C*-Ar), 113.0 (*C*-Ar), 118.8 (*C*-Ar), 119.5 (*C*-Ar), 122.2 (*C*-Ar), 122.3 (*C*-Ar), 127.6 (*C*-Ar), 136.5 (*C*-Ar), 170.4 (*C*=O). IR (film) υ: 2099, 1655. ESI-HRMS: m/z calcd for $C_{20}H_{25}N_5O_4Na$ $[M+Na]^+$ 422.1799; found 422.1821.

3,6-Anhydro-2,7-dideoxy-*N*-[2-(1*H*-indol-3-yl)ethyl]-4,5-*O*-(1-methylethylidene)-7-[4-[2-[*N*-[bis(phenylmethoxy)carbonyl]]guanidinoethyl]-1*H*-1,2,3-triazol-1-yl]-L-*galacto*-heptonamide 31

To a solution of **30** (300 mg, 0.75 mmol, 1 eq.) and 28 (284 mg, 0.75 mmol, 1 eq.) in CH_2Cl_2 (4 mL) were added H_2O (4 mL), $CuSO_4$,5H_2O (8 mg, 0.04 mmol, 0.05 eq.) and sodium ascorbate (15 mg, 0.075 mmol, 0.1 eq.). After completion of the reaction, the solution mixture was diluted with water and CH_2Cl_2 (20 mL) and the layers were separated. The aqueous layer was washed with CH_2Cl_2 (2x20 mL) and the combined organic layers were dried over $MgSO_4$, filtered and the solvent was removed under reduced pressure. The compound was purified by column chromatography (silica gel, cyH/EA) to provide **31** (466 mg, 80%). White solid. *Rf;* 0.46 (EA). $[\alpha]_D$= -3.5° (c = 0.2, $CHCl_3$). Mp = 72°C. ^{1}H NMR (250 MHz, MeOH d_4): δ = 1.29 (s, 3H, C(C*H*$_3$)), 1.47 (s, 3H, C(C*H*$_3$)), 2.53-2.58 (m, 2H, *H*-2), 2.86-2.93 (m, 2H, *H*-8), 2.89 (t, 2H, $J_{\alpha,\beta}$ = 7.5 Hz, *H*-β), 3.38-3.50 (m, 2H, *H*-α), 3.78 (m, 1H, *H*-6), 3.85 (m, 1H, *H*-3), 4.11-4.19 (m, 2H, *H*-10), 4.39 (dd, 1H, J_{gem} = 14.0, $J_{6,7}$ = 8.0 Hz, *H*-7), 4.57 (dd, 1H, $J_{6,7'}$ = 4.0 Hz, *H*-7'), 4.61-4.69 (m, 2H, *H*-4, *H*-5), 5.10 (s, 2H, C*H*$_2$-Ph), 5.11 (s, 2H, C*H*$_2$-Ph), 6.94-7.08 (m, 3H, *H*-Ar), 7.23-7.39 (m, 11H, *H*-Ar), 7.53 (d, 1H, *J* = 8.0 Hz, *H*-Ar), 7.59 (s, 1H, *H*-triazole). ^{13}C NMR (62.9 MHz, MeOH d_4): δ = 24.9 (*C*H$_3$), 25.8 (*C*-8), 26.2, 26.3 (*C*H$_3$, *C*-β), 36.6 (*C*-2), 41.5 (*C*-α), 45.4 (*C*-9), 50.5 (*C*-7), 68.3 (*C*H$_2$-Ph), 69.9 (*C*H$_2$-Ph), 79.7 (*C*-3), 80.7 (*C*-6), 82.2, 82.9 (*C*-4, *C*-5), 112.3 (*C*-Ar), 113.2 (*C*-Ar), 113.8 (*C*(CH$_3$)$_2$), 119.3 (*C*-Ar), 119.6 (*C*-Ar), 122.3 (*C*-Ar), 123.5 (*C*-Ar), 128.8 (*C*-Ar), 128.9 (4 *C*-Ar), 129.5 (5 *C*-Ar), 129.6 (*C*-Ar), 129.7 (2 *C*-Ar), 136.5 (*C*-Ar), 138.1 (*C*-Ar), 138.5 (*C*-Ar), 156.7 (NH*C*OO), 161.7 (*C*=N), 164.9 (NH*C*OO), 172.9 (*C*=O). IR (film) υ: 1721, 1648, 1612. ESI-HRMS: m/z calcd for $C_{41}H_{46}N_8O_8Na$ $[M+Na]^+$ 801.3331; found 801.3316.

3,6-Anhydro-2,7-dideoxy-*N*-[2-(1*H*-indol-3-yl)ethyl]-4,5-*O*-(1-methylethylidene)-7-[4-[3-[*N*-[bis(phenylmethoxy)carbonyl]]guanidinopropyl]-1*H*-1,2,3-triazol-1-yl]-L-*galacto*-heptonamide 32

Prepared starting from **30** (300 mg, 0.75 mmol) following procedure described for **31**. Yield: 70% (415 mg). White solid. *Rf;* 0.43 (EA). $[\alpha]_D$= -5.5° (c = 0.2, $CHCl_3$). Mp = 62°C. ^{1}H NMR (400 MHz, MeOH d_4): δ = 1.28 (s, 3H, C(C*H*$_3$)), 1.46 (s, 3H, C(C*H*$_3$)), 1.83-1.90 (m, 2H, *H*-9), 2.51 (dd, 1H, J_{gem} = 15.0, $J_{2,3}$ = 5.5 Hz, *H*-2), 2.51-2.61 (m, 3H, *H*-8, *H*-2'), 2.87 (t, 2H, $J_{\alpha,\beta}$ = 7.0 Hz, *H*-β), 3.35-3.49 (m, 2H, *H*-α), 3.77-3.85 (m, 2H, *H*-3, *H*-6), 3.88-3.92 (m, 2H, *H*-10), 4.38 (dd, 1H, J_{gem} = 14.5 , $J_{6,7}$ = 8.5 Hz, *H*-7), 4.53 (dd, 1H, $J_{6,7'}$ = 4.0 Hz, *H*-7'), 4.60-4.64 (m, 2H, *H*-4, *H*-5), 5.08 (d, 1H, *J* = 12.0 Hz, C*H*$_2$-Ph), 5.09 (d, 1H, C*H*$_2$-Ph), 5.17 (s, 2H, C*H*$_2$-Ph), 6.95-7.08 (m, 3H, *H*-Ar), 7.24-7.37 (m, 11H, *H*-Ar), 7.53 (d, 1H, *J* = 8.0 Hz, *H*-Ar), 7.69 (s, 1H, *H*-triazole). ^{13}C NMR (100 MHz, MeOH d_4): *δ* = 23.4 (*C*-8), 24.8 (*C*H$_3$), 26.2 (*C*H$_3$, *C*-β), 28.9 (*C*-9), 36.6 (*C*-2), 41.5 (*C*-α), 45.4 (*C*-10), 50.4 (*C*-7), 68.2 (*C*H$_2$-Ph), 69.9 (*C*H$_2$-Ph), 79.7 (*C*-3), 80.7 (*C*-6), 82.2, 82.9 (*C*-4, *C*-5), 112.3 (*C*-Ar), 113.2 (*C*-Ar), 113.8 (*C*(CH$_3$)$_2$), 119.3 (*C*-Ar), 119.6 (*C*-Ar), 122.3 (*C*-Ar), 123.5 (*C*-Ar), 124.0 (*C*-Ar), 128.8 (*C*-Ar), 128.9 (3 *C*-Ar), 129.4 (2 *C*-Ar), 129.5 (2 *C*-Ar), 129.7 (3 *C*-Ar), 136.5 (*C*-Ar), 138.1 (*C*-Ar), 138.5 (*C*-Ar), 148.2 (*C*-Ar), 156.8 (NH*C*OO), 161.9 (*C*=N), 165.0 (NH*C*OO), 172.9 (*C*=O). IR (film) υ: 3388,

3279, 1723, 1648, 1610. ESI-HRMS: m/z calcd for $C_{42}H_{48}N_8O_8Na$ $[M+Na]^+$ 815.3487; found 815.3480.

3,6-Anhydro-2,7-dideoxy-*N*-[2-(1*H*-indol-3-yl)ethyl]-4,5-*O*-(1-methylethylidene)-7-[4-(2-guanidinoethyl)-1*H*-1,2,3-triazol-1-yl]-L-*galacto*-heptonamide 33

Prepared starting from **31** (150 mg, 0.19 mmol) following procedure described for **15**. Yield: 90% (87 mg). White solid. *Rf;* 0.0 (EA). HPLC (60/40): t_R = 5.401 min. $[\alpha]_D$= -11.9° (c = 0.2, MeOH). Mp = 138°C. ^{1}H NMR (400 MHz, MeOH-d$_4$): δ = 1.32 (s, 3H, C(C*H*$_3$)), 1.50 (s, 3H, C(C*H*$_3$)), 2.55-2.65 (m, 2H, *H*-2), 2.89-2.94 (m, 4H, *H*-8, *H*-β), 3.41-3.49 (m, 4H, *H*-9, *H*-α), 3.87-3.93 (m, 2H, *H*-3, *H*-6), 4.53 (dd, 1H, J_{gem} = 14.0, $J_{6,7}$ = 8.5 Hz, *H*-7), 4.66-4.77 (m, 3H, *H*-7', *H*-4, *H*-5), 6.99-7.11 (m, 3H, *H*-Ar), 7.35 (d, 1H, *J* = 8.0 Hz, *H*-Ar), 7.57 (d, 1H, *J* = 8.0 Hz, *H*-Ar), 7.80 (s, 1H, *H*-triazole). ^{13}C NMR (100 MHz, MeOH-d$_4$): *δ* = 24.9 (*C*H$_3$), 26.1 (*C*-8), 26.2 (*C*H$_3$, *C*-β), 36.5 (*C*-2), 41.5 (*C*-α), 41.9 (*C*-9), 50.6 (*C*-7), 79.7 (*C*-3), 80.7 (*C*-6), 82.2, 82.9 (*C*-4, *C*-5), 112.3 (*C*-Ar), 113.2 (*C*-Ar), 113.8 (*C*(CH$_3$)$_2$), 119.3 (*C*-Ar), 119.6 (*C*-Ar), 122.3 (*C*-Ar), 123.5 (*C*-Ar), 124.8 (*C*-Ar), 128.8 (*C*-Ar), 138.1 (*C*-Ar), 145.3 (*C*-Ar), 158.9 (*C*=N), 172.9 (*C*=O). IR (pellets) υ: 3327, 1652. ESI-HRMS: m/z calcd for $C_{25}H_{34}N_8O_4Na$ $[M+Na]^+$ 533.2595; found 533.2597.

3,6-Anhydro-2,7-dideoxy-*N*-[2-(1*H*-indol-3-yl)ethyl]-4,5-*O*-(1-methylethylidene)-7-[4-(3-guanidinopropyl)-1*H*-1,2,3-triazol-1-yl]-L-*galacto*-heptonamide 34

Prepared starting from **32** (150 mg, 0.19 mmol) following procedure described for **15**. Yield: 90% (90 mg). White solid. *Rf;* 0.0 (EA). HPLC (60/40): t_R = 5.587 min. $[\alpha]_D$= -11.6° (c = 0.2, MeOH). Mp = 132°C. ^{1}H NMR (250 MHz, MeOH-d$_4$): δ = 1.32 (s, 3H, C(C*H*$_3$)), 1.49 (s, 3H, C(C*H*$_3$)), 1.83-1.89 (m, 2H, *H*-9), 2.56-2.61 (m, 2H, *H*-2), 2.68 (t, 2H, $J_{8,9}$ = 8.0 Hz, *H*-8), 2.91 (t, 2H, $J_{\alpha,\beta}$ = 7.5 Hz, *H*-β), 3.11 (t, 2H, $J_{9,10}$ = 7.0 Hz, *H*-10), 3.43-3.49 (m, 2H, *H*-α), 3.86-3.95 (m, 2H, *H*-3, *H*-6), 4.51 (dd, 1H, J_{gem} = 14.5 , $J_{6,7}$ = 8.5 Hz, *H*-7), 4.68 (m, 1H, *H*-7'), 4.73-4.80 (m, 2H, *H*-4, *H*-5), 6.96-7.11 (m, 3H, *H*-Ar), 7.33 (d, 1H, *J* = 8.0 Hz, *H*-Ar), 7.55 (d, 1H, *J* = 8.0 Hz, *H*-Ar), 7.73 (s, 1H, *H*-triazole). ^{13}C NMR (62.9 MHz, MeOH-d$_4$): δ = 23.2 (*C*-8), 24.9 (*C*H$_3$), 26.2 (*C*H$_3$, *C*-β), 29.6 (*C*-9), 36.6 (*C*-2), 41.5 (*C*-α), 41.7 (*C*-10), 50.6 (*C*-7), 79.8 (*C*-3), 80.8 (*C*-6), 82.3, 83.0 (*C*-4, *C*-5), 112.3 (*C*-Ar), 113.3 (*C*-Ar), 113.9 (*C*(CH$_3$)$_2$), 119.3 (*C*-Ar), 119.7 (*C*-Ar), 122.4 (*C*-Ar), 123.6 (*C*-Ar), 124.2 (*C*-Ar), 128.8 (*C*-Ar), 138.2 (*C*-Ar), 147.8 (*C*-Ar), 159.0 (*C*=N), 173.0 (*C*=O). IR (pellets) υ: 3355, 1650. ESI-HRMS: m/z calcd for $C_{26}H_{36}N_8O_4Na$ $[M+Na]^+$ 547.2752; found 547.2752.

3,6-Anhydro-2,7-dideoxy-*N*-[2-(1*H*-indol-3-yl)ethyl]-7-[4-[2-[*N*-[bis(phenylmethoxy)carbonyl]]guanidinoethyl]-1*H*-1,2,3-triazol-1-yl]-L-*galacto*-heptonamide 35

To a stirred solution of **31** (250 mg, 0.32 mmol) in H_2O (2 ml) at 0°C was added trifluoroacetic acid (2 ml). After completion of the reaction (tlc monitoring), the solution mixture was diluted with EtOAc (50 mL) and $NaHCO_3$ sat. aq. was added until pH 5-6. The product was extracted with EtOAc (2x15 ml) and the combined organic layers were dried over $MgSO_4$, filtered and the solvent was removed under reduced pressure. The compound was purified by column chromatography (silica gel, EA/MeOH) to provide **35** (200 mg, 85%). Colorless gum. *Rf;* 0.23 (EA/MeOH: 9/1). $[\alpha]_D$= -5.8° (c = 0.1, MeOH). ^{1}H NMR (250 MHz, MeOH-d$_4$): δ = 2.46-2.58 (m, 2H, *H*-2), 2.89 (t, 4H, *H*-8, *H*-β), 3.46 (t, 2H, $J_{\alpha,\beta}$ = 7.0 Hz, *H*-α), 4.01-4.18 (m, 5H, *H*-4, *H*-5, *H*-6, *H*-9), 4.30 (t, 1H, $J_{2,3}$ = 5.5 Hz, *H*-3), 4.41 (dd, 1H, J_{gem} = 14.5, $J_{6,7}$ = 9.0 Hz, *H*-7), 4.56 (dd, 1H, $J_{6,7'}$ = 3.5 Hz, *H*-7'), 5.08 (s, 3H, *C*H$_2$-Ph), 5.11 (s, 3H, *C*H$_2$-Ph), 6.92-7.08 (m, 3H, *H*-Ar), 7.25-7.37 (m, 11H, *H*-Ar), 7.52 (d, 1H, *J* = 8.0 Hz, *H*-Ar), 7.58 (s, 1H, *H*-triazole). ^{13}C NMR (62.9 MHz, MeOH-d$_4$): *δ* = 25.8, 26.3 (*C*-8, *C*-β), 38.4 (*C*-2), 41.5 (*C*-α), 45.4 (*C*-9), 52.7 (*C*-7), 68.4, 69.9 (*C*H$_2$-Ph), 73.3 (*C*-4, *C*-5), 79.0 (*C*-3), 79.9 (*C*-6), 112.3 (*C*-Ar), 113.2

(*C*-Ar), 119.3 (*C*-Ar), 119.6 (*C*-Ar), 122.3 (*C*-Ar), 123.4 (*C*-Ar), 124.6 (*C*-Ar), 128.9 (4 *C*-Ar), 129.4 (4 *C*-Ar), 129.7 (4 *C*-Ar), 136.4 (*C*-Ar), 138.1 (*C*-Ar), 138.5 (*C*-Ar), 156.7 (*C*=N), 161.7 (2 NH*C*OO), 162.9, 163.2 (TFA), 173.9 (*C*=O). IR (pellets) υ: 3325, 1665, 1632. ESI-HRMS: m/z calcd for $C_{38}H_{42}N_8O_8Na$ $[M+Na]^+$ 761.3018; found 761.3011.

3,6-Anhydro-2,7-dideoxy-*N*-[2-(1*H*-indol-3-yl)ethyl]-7-[4-[3-[N-[bis(phenylmethoxy)carbonyl]]guanidinopropyl]-1*H*-1,2,3-triazol-1-yl]-L-*galacto*-heptonamide 36

Prepared starting from **32** (250 mg, 0.32 mmol) following procedure described for **35**. Yield: 53% (127 mg). Colorless gum. *Rf;* 0.34 (EA/MeOH: 9/1). $[\alpha]_D$= -2.0° (c = 0.2, MeOH). ^{1}H NMR (250 MHz, MeOH-d$_4$): δ = 1.82-1.93 (m, 2H, *H*-9), 2.47-2.61 (m, 4H, *H*-2, *H*-8), 2.85-2.90 (m, 2H, *H*-β), 3.44 (t, 2H, $J_{\alpha,\beta}$ = 7.0 Hz, *H*-α), 3.89 (t, 2H, $J_{9,10}$ = 7.5 Hz, *H*-10), 4.04-4.15 (m, 3H, *H*-4, *H*-5, *H*-6), 4.29 (t, 1H, $J_{2,3}$ = 5.5 Hz, *H*-3), 4.42 (dd, 1H, J_{gem} = 14.5, $J_{6,7}$ = 9.0 Hz, *H*-7), 4.54 (dd, 1H, $J_{6,7'}$ = 3.5 Hz, *H*-7'), 5.11 (s, 2H, *C*H$_2$-Ph), 5.18 (s, 2H, *C*H$_2$-Ph), 6.92-7.07 (m, 3H, *H*-Ar), 7.25-7.38 (m, 11H, *H*-Ar), 7.52 (d, 1H, *J* = 8.0 Hz, *H*-Ar), 7.69 (s, 1H, *H*-triazole). ^{13}C NMR (62.9 MHz, MeOH-d$_4$): δ = 23.3 (*C*-9), 26.3, 28.7 (*C*-8, *C*-β), 38.5 (*C*-2), 41.5 (*C*-α), 45.8 (*C*-10), 52.7 (*C*-7), 68.5, 70.1 (*C*H$_2$-Ph), 73.3 (*C*-4, *C*-5), 79.0 (*C*-3), 79.9 (*C*-6), 112.2 (*C*-Ar), 113.2 (*C*-Ar), 119.3 (*C*-Ar), 119.6 (*C*-Ar), 122.3 (*C*-Ar), 123.5 (*C*-Ar), 124.2 (*C*-Ar), 128.7 (*C*-Ar), 129.0 (3 *C*-Ar), 129.4 (2 *C*-Ar), 129.5 (2 *C*-Ar), 129.7 (4 *C*-Ar), 136.4 (*C*-Ar), 138.1 (*C*-Ar), 138.2 (*C*-Ar), 156.5 (*C*=N), 161.4 (2 NH*C*OO), 163.0 (TFA), 173.9 (*C*=O). IR (pellets) υ: 3368, 1668, 1645. ESI-HRMS: m/z calcd for $C_{39}H_{44}N_8O_8Na$ $[M+Na]^+$ 775.3174; found 775.3172.

3,6-anhydro-2,7-dideoxy-*N*-[2-(1*H*-indol-3-yl)ethyl]-7-[4-(3-guanidinoethyl)-1*H*-1,2,3-triazol-1-yl]-L-*galacto*-heptonamide 37

Prepared starting from **35** (150 mg, 0.2 mmol) following procedure described for **15**. Yield: 80% (75 mg). Colorless gum. *Rf;* 0.58 (H$_2$O/MeCN/TFA: 4/6/0.1%). HPLC (60/40): t_R = 3.678 min. $[\alpha]_D$= -9.0° (c = 0.1, MeOH). ^{1}H NMR (250 MHz, MeOH-d$_4$): δ = 2.56-2.61 (m, 2H, *H*-2), 2.85-2.96 (m, 4H, *H*-8, *H*-β), 3.40-3.51 (m, 4H, *H*-α, *H*-9), 4.11-4.19 (m, 3H, *H*-4, *H*-5, *H*-6), 4.37 (t, 1H, $J_{2,3}$ = 5.5 Hz, *H*-3), 4.52 (dd, 1H, J_{gem} = 14.0, $J_{6,7}$ = 9.0 Hz, *H*-7), 4.65 (dd, 1H, $J_{6,7'}$ = 3.0 Hz, *H*-7'), 6.96-7.11 (m, 3H, *H*-Ar), 7.32 (d, 1H, *J* = 8.0 Hz, *H*-Ar), 7.55 (d, 1H, *J* = 8.0 Hz, *H*-Ar), 7.77 (s, 1H, *H*-triazole). ^{13}C NMR (62.9 MHz, MeOH-d$_4$): δ = 24.9, 25.1 (*C*-8, *C*-β), 37.3 (*C*-2), 40.4 (*C*-α), 40.7 (*C*-9), 51.7 (*C*-7), 72.2 (*C*-4, *C*-5), 78.0 (*C*-3), 78.8 (*C*-6), 111.1 (*C*-Ar), 112.1 (*C*-Ar), 118.1 (*C*-Ar), 118.4 (*C*-Ar), 121.2 (*C*-Ar), 122.3 (2 *C*-Ar), 123.9 (*C*-Ar), 127.6 (*C*-Ar), 137.0 (*C*-Ar), 157.5 (*C*=N), 172.7 (*C*=O). IR (pellets) υ: 3339, 3210, 2657, 2922, 1665, 1649. ESI-HRMS: m/z calcd for $C_{22}H_{30}N_8O_4Na$ $[M+Na]^+$ 493.2282; found 493.2274.

3,6-anhydro-2,7-dideoxy-*N*-[2-(1*H*-indol-3-yl)ethyl]-7-[4-(3-guanidinopropyl)-1*H*-1,2,3-triazol-1-yl]-L-*galacto*-heptonamide 38

Prepared starting from **36** (100 mg, 0.13 mmol) following procedure described for **15**. Yield: 95% (60 mg). Colorless gum. *Rf;* 0.34 (H$_2$O/MeCN/TFA: 6/4/0.1%). HPLC (60/40): t_R = 3.692 min. $[\alpha]_D$= -6.5° (c = 0.2, MeOH). ^{1}H NMR (250 MHz, D$_2$O): δ = 1.63-1.75 (m, 2H, *H*-9), 2.38-2.59 (m, 4H, *H*-2*,* *H*-8), 2.81-2.94 (m, 4H, *H*-10, *H*-β), 3.33-3.51 (m, 2H, *H*-α), 4.01-4.21 (m, 3H, *H*-4, *H*-5, *H*-6), 4.33-4.45 (m, 2H, *H*-3, *H*-7), 4.54 (dd, 1H, J_{gem} = 14.5, $J_{6,7'}$ = 3.0 Hz, *H*-7'), 6.98-7.18 (m, 3H, *H*-Ar), 7.39 (d, 1H, *J* = 8.0 Hz, *H*-Ar), 7.54-7.57 (m, 2H, *H*-Ar, *H*-triazole). ^{13}C NMR (62.9 MHz, D$_2$O): δ = 21.7 (*C*-9), 24.2, 27.4 (*C*-8, *C*-β), 37.0 (*C*-2), 40.3 (*C*-α, *C*-10), 51.3 (*C*-7), 71.6, 71.8 (*C*-4, *C*-5), 77.2 (*C*-3), 77.9 (*C*-6), 111.7 (*C*-Ar), 111.8 (*C*-Ar), 118.4 (*C*-Ar), 119.1 (*C*-Ar), 121.8 (*C*-Ar), 123.3 (2 *C*-Ar), 123.7 (*C*-Ar), 126.9 (*C*-Ar),

136.1 (*C*-Ar), 156.5 (*C*=N), 173.5 (*C*=O). IR (pellets) υ: 3279, 1622, 1549. ESI-HRMS: m/z calcd for $C_{23}H_{32}N_8O_4Na$ $[M+Na]^+$ 507.2439; found 507.2445.

N^{α}-[2-*C*-[2,3:5,6-Bis-*O*-(1-methylethylidene)-α-D-gulofuranosyl]carboxymethyl)]-L-nitro-arginine methyl ester 39

To a solution of N^{ω}-NO_2-L-arg-OMe (296 mg, 1.1 mmol, 1.1 eq.) and **5** (300 mg, 1 mmol, 1 eq.) in DMF (10 mL) were added HATU (570 mg, 1.5 mmol, 1.5 eq.) and DIEA (363 µL, 2.1 mmol, 2.1 eq.) at 0°C under argon atmosphere. After stirring at room temperature for 24h, the solvent was evaporated. The residue was dissolved in ethyl acetate (10 mL), washed with 1N HCl (5 mL), $NaHCO_3$ sat. aq. (5 mL) and brine (5 mL). The organic layer was dried over $MgSO_4$, filtered and concentrated. The product was purified by column chromatography (silica gel, cyclohexane/AcOEt) to provide **39** (258 mg, 50%). Colorless oil. *Rf;* 0.36 (CH_2Cl_2/MeOH: 9/1). $[\alpha]_D$= -22.2° (c = 0.2, $CHCl_3$). ^{1}H NMR (250 MHz, DMSO-d_6): δ = 1.20 (s, 3H, C(C*H*$_3$)$_2$), 1.25 (s, 3H, C(C*H*$_3$)$_2$), 1.30 (s, 3H, C(C*H*$_3$)$_2$), 1.36 (s, 3H, C(C*H*$_3$)$_2$), 1.45-1.73 (m, 4H, C*H*$_2$ *arg*), 2.49-2.54 (m, 2H, *H*-2), 3.10-3.17 (m, 2H, C*H*$_2$ *arg*), 3.41 (dd, 1H, $J_{6,7}$ = 8.5, $J_{5,6}$ = 3.5 Hz, *H*-6), 3.62 (s, 3H, OC*H*$_3$), 3.66 (t, 1H, $J_{7,8}$ = 7.5 Hz, *H*-8), 3.85 (td, 1H, $J_{2,3}$ = 6.5, $J_{3,4}$ = 3.0 Hz, *H*-3), 4.01 & 4.01 (2m, 2H, *H*-7, *H*-8'), 4.26 (m, 1H, *H*-α), 4.60 (dd, 1H, $J_{4,5}$ = 6.0 Hz, *H*-4), 4.66 (dd, 1H, *H*-5), 7.91 (br s, 2H, N*H*), 8.32 (d, 1H, *J* = 7.5 Hz, N*H*), 8.46 (br s, 1H, N*H*). ^{13}C NMR (62.9 MHz, DMSO-d_6): δ = 24.7 (*C*H$_3$, *C*H$_2$ *arg*), 25.3 (*C*H$_3$), 25.8 (*C*H$_3$), 26.5 (*C*H$_3$), 28.2 (C*H*$_2$ *arg*), 34.3 (*C*-2), 40.1 (C*H*$_2$ *arg*), 51.5 (*C*-α), 51.8 (O*C*H$_3$), 65.2 (*C*-8), 75.2 (*C*-7), 77.4 (*C*-3), 80.6 (*C*-5), 81.1 (*C*-4), 82.4 (*C*-6), 108.6 (*C*(CH$_3$)$_2$), 111.3 (*C*(CH$_3$)$_2$), 159.3 (*C*=N), 169.7 (*C*=O), 172.5 (*C*=O). IR (film) υ: 3317, 2985, 2928, 2862, 1742, 1650, 1629, 1598, 1539. ESI-HRMS: m/z calcd for $C_{21}H_{35}N_5O_{10}Na$ $[M+Na]^+$ 540.2276; found 540.2294.

N^{α}-[2-*C*-[2,3:5,6-Bis-*O*-(1-methylethylidene)-α-D-gulofuranosyl]carboxymethyl)]-L-arginine 40

To a solution of **39** (200 mg, 0.39 mmol) in THF (9 mL) and water (3 mL) was added LiOH (30 mg, 1.17 mmol, 3 eq.) and the mixture was stirred until completion of the reaction monitored by tlc. Amberlite® IR-120 was added until pH = 3-4 and the mixture was filtered off. The solvent were evaporated and the obtained carboxylic acid was solubilized in MeOH (10 mL) and Pd/C (10%) (80 mg, 40% w/w) was added. After stirring under H_2 atmosphere (40 psi) until completion of the reaction, the mixture was filtered through a pad of celite and the filtrate was concentrated *in vacuo*. The crude was purified by column chromatography (C-18 silica gel, H_2O/MeOH) and lyophilized to provide **40** (125 mg, 70%). White foam. *Rf;* 0.32 (H_2O/MeOH: 1/1). HPLC (70/30): t_R = 4.436 min. $[\alpha]_D$= -1.0° (c = 0.2, H_2O). ^{1}H NMR (400 MHz, D_2O): δ = 1.26 (s, 3H, C(C*H*$_3$)$_2$), 1.32 (s, 3H, C(C*H*$_3$)$_2$), 1.38 (s, 3H, C(C*H*$_3$)$_2$), 1.42 (s, 3H, C(C*H*$_3$)$_2$), 1.51-1.60 (m, 2H, C*H*$_2$ *arg*, 1.66 (m, 1H, C*H*$_2$ *arg*), 1.77 (m, 1H, C*H*$_2$ *arg*), 2.70 (d, 2H, $J_{2,3}$ = 7.0 Hz, *H*-2), 3.12 (t, 2H, *J* = 7.0 Hz, C*H*$_2$ *arg*), 3.64 (dd, 1H, $J_{6,7}$ = 8.5, $J_{5,6}$ = 3.5 Hz, *H*-6), 3.80 (dd, 1H, J_{gem} = 9.0, $J_{7,8}$ = 6.5 Hz, *H*-8), 3.99 (td, 1H, $J_{3,4}$ = 3.5 Hz, *H*-3), 4.12 (dd, 1H, *J* = 8.0, *J* = 5.0 Hz, *H*-α), 4.17 (dd, 1H, $J_{7,8'}$ = 7.0 Hz, *H*-8'), 4.33 (m, 1H, *H*-7), 4.77 (dd, 1H, $J_{4,5}$ = 6.0 Hz, *H*-4), 4.81 (dd, 1H, *H*-5). ^{13}C NMR (100 MHz, D_2O): δ = 23.6 (*C*H$_3$), 24.1 (*C*H$_3$), 24.4 (*C*H$_2$ *arg*), 24.7 (*C*H$_3$), 25.5 (*C*H$_3$), 28.7(*C*H$_2$ *arg*), 34.6 (*C*-2), 40.6 (*C*H$_2$ *arg*), 54.6 (*C*-α), 65.2 (*C*-8), 74.6 (*C*-7), 78.0 (*C*-3), 80.4 (*C*-5), 81.1 (*C*-4), 82.6 (*C*-6), 110.4 (*C*(CH$_3$)$_2$), 113.1 (*C*(CH$_3$)$_2$), 156.7 (*C*=N), 171.8 (*C*=O), 178.6 (*C*=O). IR (pellets) υ: 3364, 2985, 2938, 2867, 1652, 1579. ESI-HRMS: m/z calcd for $C_{20}H_{34}N_4O_8Na$ $[M+Na]^+$ 481.2269; found 481.2275.

(*E*)-3,6-Anhydro-2-deoxy-4,5:7,8-bis-*O*-(1-methylethylidene)-D-*gulo*-Oct-2-enonic acid methyl 41

To a solution of **2** (630 mg, 2 mmol, 1 eq.) in pyridine (15 mL) was added LiI (2.6 g, 20 mmol, 10 eq.) and the mixture was stirred at 120 °C until completion of the reaction monitored

by tlc. The solvent was removed under reduced pressure, CH_2Cl_2 (20 mL) was added and the pH was adjusted to 2-3 with 1N HCl. The layers were separated and the organic layer was washed with brine and dried over $MgSO_4$, filtered and concentrated. The crude residue was purified by column chromatography (silica gel, cyH/EA) to provide **41** (300 mg, 50%). White solid. *Rf;* 0.21 (cH/EA: 1/1). $[\alpha]_D$= -36.5° (c = 1.0, $CHCl_3$). Mp = 201°C. ^{1}H NMR (250 MHz, $CDCl_3$): δ = 1.37 (s, 3H, C*H*$_3$), 1.39 (s, 3H, C*H*$_3$), 1.43 (s, 3H, C*H*$_3$), 1.46 (s, 3H, C*H*$_3$), 3.77 (dd, 1H, J_{gem} = 9.0, $J_{7,8}$ = 7.0 Hz, *H*-8), 4.18-4.26 (m, 2H, *H*-6, *H*-8), 4.42 (m, 1H, *H*-7), 4.75 (dd, 1H, $J_{4,5}$ = 6.0, $J_{5,6}$ = 4.0 Hz, *H*-5), 5.51 (s, 1H, *H*-2), 5.76 (d, 1H, *H*-4), 10.66 (br s, 1H, COO*H*). ^{13}C NMR (62.9 MHz, $CDCl_3$): δ = 25.2 (*C*H$_3$), 25.6 (*C*H$_3$), 26.4 (*C*H$_3$), 26.7 (*C*H$_3$), 65.7 (*C*-8), 75.5, 77.5, 79.9, 86.5 (*C*-4, *C*-5, *C*-6, *C*-7), 94.9 (*C*-2), 110.3, 113.7 (2 *C*(CH$_3$)$_2$), 172.0 (*C*-3), 173.1 (*C*=O). IR (film) υ: 3061, 2985, 2933, 2563, 1685, 1655. ESI-HRMS: m/z calcd for $C_{14}H_{20}O_7Na$ $[M+Na]^+$ 323.1101; found 323.1102.

Compound 42

To a solution of HCl.H-L-arg-OMe (191 mg, 0.73 mmol, 1.1 eq.) and 41 (200 mg, 0.67 mmol, 1 eq.) in DMF (10 mL) were added HATU (380 mg, 1.0 mmol, 1.5 eq.) and DIEA (243 µL, 1.41 mmol, 2.1 eq.) at 0°C under argon atmosphere. After stirring at room temperature for 24h, the solvent was evaporated. The crude product was solubilized in pyridine (15 mL), LiI (871 mg, 6.7 mmol, 10 eq.) was added and the mixture was stirred at 120 °C until completion of the reaction monitored by tlc. The solvent was removed under reduced pressure and the crude was purified by column chromatography (C-18 silica gel, H_2O/MeOH) and lyophilized to provide **42** (96 mg, 33%). White solid. *Rf;* 0.41 (H_2O/MeOH: 1/1). HPLC (70/30): t_R = 4.782 min. $[\alpha]_D$= -44.6° (c = 0.08, H_2O). ^{1}H NMR (250 MHz, D_2O): δ = 1.47 (s, 3H, C*H*$_3$), 1.48 (s, 3H, C*H*$_3$), 1.53 (s, 3H, C*H*$_3$), 1.54 (s, 3H, C*H*$_3$), 1.63-1.75 (m, 2H, C*H*$_2$ *arg*), 1.79-1.99 (m, 2H, C*H*$_2$ *arg*), 3.26 (t, 2H, *J* = 7.0 Hz, C*H*$_2$ *arg*), 4.08 (dd, 1H, J_{gem} = 9.0, $J_{7,8}$ = 6.0 Hz, *H*-8), 4.32-4.39 (m, 2H, *H*-6, *H*-8'), 4.62 (m, 1H, *H*-7), 4.73 (m, 1H, *H*-α), 5.07 (dd, 1H, $J_{4,5}$ = 6.0, $J_{5,6}$ = 4.0 Hz, *H*-5), 5.22 (d, 1H, $J_{2,4}$ = 1.0 Hz, *H*-2), 5.49 (dd, 1H, *H*-4). ^{13}C NMR (62.9 MHz, D_2O): δ = 24.6 (*C*H$_3$, *C*H$_2$ *arg*), 25.0 (*C*H$_3$), 25.9 (*C*H$_3$), 26.0 (*C*H$_3$), 29.4 (*C*H$_2$ *arg*), 41.0 (*C*H$_2$ *arg*), 54.6 (*C*-α), 65.6 (*C*-8), 74.9 (*C*-7), 77.2 (*C*-5), 81.6 (*C*-4), 87.0 (*C*-6), 96.0 (*C*-2), 111.0 (*C*(CH$_3$)$_2$), 115.2 (*C*(CH$_3$)$_2$), 157.0 (*C*=N), 165.5 (*C*-3), 167.3 (*C*=O), 178.9 (*C*=O). IR (pellets) υ: 3132, 3165, 2981, 2937, 2725, 2860, 1672, 1583. ESI-HRMS: m/z calcd for $C_{20}H_{33}N_4O_8$ $[M+H]^+$ 457.2293; found 457.2278.

3,6-Anhydro-2-deoxy-4,5-*O*-(1-methylethylidene)-D-*glycero*-L-*galacto*-octonic acid methyl ester 43

To a stirred solution of **4** (3.16 g, 10.0 mmol) in methanol (200 mL) at 0°C was added an aqueous solution of 1N HCl (70 mL). After stirring at room temperature until completion of the reaction, sat. aq. $NaHCO_3$ was added until pH = 7. Half of the solvent was removed under vacuo and the product was extracted with CH_2Cl_2 (3 x 100 mL). The organic layer was dried over $MgSO_4$, filtered and the solvent was evaporated. The crude residue was purified by column chromatography (silica gel, cyH/EA) to provide **43** (2.54 g, 92%). White solid. *Rf;* 0.29 (EA). $[\alpha]_D$= -5.6° (c = 1, $CHCl_3$). Mp = 50°C. ^{1}H NMR (250 MHz, $CDCl_3$): δ = 1.31 (s, 3H, C(C*H*$_3$)$_2$), 1.47 (s, 3H, C(C*H*$_3$)$_2$), 2.78 (dd, 1H, J_{gem} = 17.0, $J_{2,3}$ = 7.0 Hz, *H*-2), 2.86 (dd, 1H, $J_{2',3}$ = 7.0 Hz, *H*-2'), 3.57 (dd, 1H, $J_{6,7}$ = 6.5, $J_{5,6}$ = 3.0 Hz, *H*-6), 3.72 (s, 3H, O-C*H*$_3$), 3.74 (dd, 1H, J_{gem} = 11.5, $J_{7,8}$ = 5.0 Hz, *H*-8), 3.81 (dd, 1H, $J_{7,8'}$ = 4.0 Hz, *H*-8'), 3.95 (td, 1H, $J_{3,4}$ = 3.5 Hz, *H*-3), 4.09 (m, 1H, *H*-7), 4.75 (dd, 1H, $J_{4,5}$ = 6.0 Hz, *H*-4), 4.79 (dd, 1H, *H*-5). ^{13}C NMR (62.9 MHz, $CDCl_3$): δ = 24.6 (*C*H$_3$), 25.7 (*C*H$_3$), 33.3 (*C*-2), 51.8 (O*C*H$_3$), 63.4 (*C*-7), 70.7 (*C*-8), 77.3 (*C*-3), 81.0, 81.3 (*C*-4, *C*-5, *C*-6), 112.6 (*C*(CH$_3$)$_2$), 171.5 (*C*=O). IR (film) υ: 3440, 2990, 2938, 2876, 1735. ESI-HRMS: m/z calcd for $C_{12}H_{20}O_7K$ $[M+K]^+$ 315.0841; found 315.0858.

3,6-Anhydro-2-deoxy-4,5-*O*-(1-methylethylidene)-L-*galacto*-heptonic acid methyl ester 44

To a stirred solution of **43** (2.2 g, 7.97 mmol) in methanol (180 mL) was added $NaIO_4$ (3.42 g, 16.0 mmol, 2 eq.) under argon. After stirring at room temperature until completion of the reaction, the solvent was removed by half *in vacuo*. The mixture was diluted with CH_2Cl_2 (200 mL). The organic layer was washed with water (3 x 75 mL), dried over $MgSO_4$, filtered and the solvent was removed under reduced pressure. The product was used without further purification.To a stirred solution of the obtained aldehyde in MeOH (190 mL) was added $NaBH_4$ (305 mg, 7.97 mmol, 1 eq.) at 0°C. After 1 h at room temperature, the solvent was removed under reduced pressure. The residue was diluted in CH_2Cl_2 (100 mL) and the organic layer was washed with a solution of 1N HCl (30 mL) and with water until pH = 7. The organic layer was dried over $MgSO_4$, filtrered and evaporated under reduced pressure. The crude product **44** was used without further purification. Yield: 93% (1.82 g). Colorless oil. *Rf;* 0.47 (EA). $[\alpha]_D$= -1.4° (c = 0.2, $CHCl_3$). ^{1}H NMR (250 MHz, $CDCl_3$): δ = 1.32 (s, 3H, C(C*H*$_3$)$_2$), 1.47 (s, 3H, C(C*H*$_3$)$_2$), 1.94 (br s, 1H, O*H*), 2.77 (dd, 1H, J_{gem} = 17.0, $J_{2,3}$ = 7.0 Hz, *H*-2), 2.85 (dd, 1H, $J_{2',3}$ = 7.0 Hz, *H*-2'), 3.66 (m, 1H, H-6), 3.71 (s, 3H, C*H*$_3$), 3.89 (dd, 1H, J_{gem} = 11.0, $J_{6,7}$ = 5.0 Hz, *H*-7), 3.96 (dd, 1H, $J_{6,7'}$ =4.0 Hz, *H*-7'), 3.97 (m, 1H, *H*-3), 4.75 (dd, 1H, $J_{4,5}$ = 6.0 Hz, *H*-4), 4.79 (dd, 1H, *H*-5). ^{13}C NMR (62.9 MHz, $CDCl_3$): δ = 24.7 (*C*H$_3$), 25.8 (*C*H$_3$), 33.3 (*C*-2), 51.8 (O*C*H$_3$), 61.0 (*C*-7), 77.4 (*C*-3), 80.9, 81.3, 81.4 (*C*-4, *C*-5, *C*-6), 112.7 (*C*(CH$_3$)$_2$), 171.4 (*C*=O). IR (film) υ: 3459, 2985, 2928, 2862, 1737. ESI-HRMS: m/z calcd for $C_{11}H_{18}O_6Na$ $[M+Na]^+$ 269.0996; found 269.1030.

3,6-Anhydro-2-deoxy-4,5-*O*-(1-methylethylidene)-7-(4-methylbenzenesulfonate)-L-*galacto*-heptonic acid methyl ester 45

Prepared starting from **44** (1.50 g, 6.1 mmol) following procedure described for **8**. Yield: 92% (2.24 g). Colorless oil. *Rf;* 0.75 (EA). $[\alpha]_D$= + 4.5° (c = 0.2, $CHCl_3$). ^{1}H NMR (250 MHz, $CDCl_3$): δ = 1.26 (s, 3H, C(C*H*$_3$)$_2$), 1.34 (s, 3H, C(C*H*$_3$)$_2$), 2.45 (s, 3H, Ph-C*H*$_3$), 2.68 (dd, 1H, J_{gem} = 16.0, $J_{2,3}$ = 7.0 Hz, *H*-2), 2.78 (dd, 1H, $J_{2',3}$ = 7.0 Hz, *H*-2'), 3.70 (s, 3H, OC*H*$_3$), 3.76 (m, 1H, *H*-6), 3.91 (td, 1H, $J_{3,4}$ = 3.5 Hz, *H*-3), 4.17 (dd, 1H, J_{gem} = 10.5, $J_{6,7}$ = 7.0 Hz, *H*-7), 4.31 (dd, 1H, $J_{6,7'}$ = 5.0 Hz, *H*-7'), 4.67 (dd, 1H, $J_{4,5}$ = 6.0 Hz, *H*-4), 4.73 (dd, 1H, $J_{5,6}$ = 3.5 Hz, *H*-5), 7.34 (d, 2H, *J* = 8.0 Hz, *H*-Ar), 7.81 (d, 2H, *J* = 8.0 Hz, *H*-Ar). ^{13}C NMR (62.9 MHz, $CDCl_3$): δ = 21.6 (Ph-*C*H$_3$), 24.8 (*C*H$_3$), 25.7 (*C*H$_3$), 33.2 (*C*-2), 51.8 (O*C*H$_3$), 67.5 (*C*-7), 77.7 (*C*-3), 78.5 (*C*-6), 80.5, 80.9 (*C*-4, *C*-5), 112.8 (*C*(CH$_3$)$_2$), 128.1 (2 *C*-Ar), 129.7 (2 *C*-Ar), 132.8 (*C*-Ar), 144.8 (*C*-Ar), 171.2 (*C*=O). IR (film) υ: 2981, 2928, 2853, 1735, 1596. ESI-HRMS: m/z calcd for $C_{18}H_{24}O_8SNa$ $[M+Na]^+$ 423.1084; found 423.1116.

3,6-Anhydro-2,7-dideoxy-4,5-*O*-(1-methylethylidene)-7-azido-L-*galacto*-heptonic acid methyl ester 46

Prepared starting from **45** (2.0 g, 5.0 mmol) following procedure described for **30**. Yield: 84% (1.14 g). Colorless oil. *Rf;* 0.51 (cH/EA: 1/1). $[\alpha]_D$= + 6.0° (c = 0.1, $CHCl_3$). ^{1}H NMR (250 MHz, $CDCl_3$): δ = 1.32 (s, 3H, C(C*H*$_3$)$_2$), 1.46 (s, 3H, C(C*H*$_3$)$_2$), 2.76 (dd, 1H, J_{gem} = 17.0, $J_{2,3}$ = 7.0 Hz, *H*-2), 2.84 (dd, 1H, $J_{2',3}$ = 7.0 Hz, *H*-2'), 3.51 (dd, 1H, J_{gem} = 12.5, $J_{6,7}$ = 6.5 Hz, *H*-7), 3.57 (dd, 1H, $J_{6,7'}$ = 6.5 Hz, *H*-7'), 3.67 (m, 1H, H-6), 3.71 (s, 3H, OC*H*$_3$), 3.96 (td, 1H, $J_{3,4}$ = 3.5 Hz, *H*-3), 4.70 (dd, 1H, $J_{4,5}$ = 6.0 Hz, *H*-4), 4.78 (dd, 1H, $J_{5,6}$ = 3.5 Hz, *H*-5). ^{13}C NMR (62.9 MHz, $CDCl_3$): δ = 25.1 (*C*H$_3$), 26.0 (*C*H$_3$), 33.5 (*C*-2), 49.8 (*C*-7), 52.0 (O*C*H$_3$), 77.9 (*C*-3), 79.9 (*C*-6), 81.0, 81.3 (*C*-4, *C*-5), 113.0 (*C*(CH$_3$)$_2$), 171.5 (*C*=O). IR (film) υ: 2985, 2943, 2867, 2099, 1742. ESI-HRMS: m/z calcd for $C_{11}H_{17}N_3O_5Na$ $[M+Na]^+$ 294.1060; found 294.1065.

3,6-anhydro-2,7-dideoxy-4,5-*O*-(1-methylethylidene)-7-benzosulfonylamino-L-*galacto*-heptonic acid methyl ester 47

To a solution of **46** (500 mg, 1.84 mmol, 1 eq.) in MeOH (10 mL) was added Pd/C (10%) (50 mg, 10% w/w). After stirring under H_2 atmosphere (15 psi) until completion of the reaction, the mixture was filtered through a pad of celite and the filtrate was concentrated *in vacuo*. The crude amine was solubilized in pyridine (3 mL) and this solution was added dropwise under argon atmosphere to a stirred solution of benzylsulfonyl chloride (282 µL, 2.21 mmol, 1.2 eq.) in pyridine (6 mL). After stirring at room temperature until completion of the reaction, the solvent was removed under reduced pressure. The residue was diluted with EtOAc (15 mL) and washed with H_2O (2x5 mL). The organic layer was dried over $MgSO_4$, filtered and the solvent was evaporated under reduced pressure. The compound was purified by flash chromatography (silica gel, cyH/EA) to provide **47** (566 mg, 80%). Colorless oil. *Rf;* 0.22 (cH/EA: 7/3). $[\alpha]_D$= +12.2° (c = 0.5, $CHCl_3$). ^{1}H NMR (250 MHz, $CDCl_3$): δ = 1.27 (s, 3H, C(C*H*$_3$)$_2$), 1.36 (s, 3H, C(C*H*$_3$)$_2$), 2.64 (dd, 1H, J_{gem} = 16.5, $J_{2,3}$ = 6.5 Hz, *H*-2), 2.73 (dd, 1H, $J_{2',3}$ = 6.5 Hz, *H*-2'), 3.27 (t, 2H, $J_{7,NH}$ = 6.5, $J_{6,7}$ = 6.5 Hz, *H*-7), 3.63 (td, 1H, $J_{5,6}$ = 3.5 Hz, *H*-6), 3.70 (s, 3H, OC*H*$_3$), 3.88 (td, 1H, $J_{3,4}$ = 3.5 Hz, *H*-3), 4.63 (dd, 1H, $J_{4,5}$ = 6.0 Hz, *H*-4), 4.71 (dd, 1H, *H*-5), 4.88 (t, 1H, N*H*), 7.48-7.61 (m, 3H, *H*-Ar), 7.85-7.89 (m, 2H, *H*-Ar). ^{13}C NMR (62.9 MHz, $CDCl_3$): δ = 24.7 (*C*H$_3$), 25.7 (*C*H$_3$), 33.3 (*C*-2), 42.1 (*C*-7), 51.8 (O*C*H$_3$), 77.5 (*C*-3), 79.1 (*C*-6), 80.8, 81.2 (*C*-4, *C*-5), 112.7 (*C*(CH$_3$)$_2$), 127.1 (2 *C*-Ar), 129.1 (2 *C*-Ar), 132.6 (*C*-Ar), 140.0 (*C*-Ar), 171.2 (*C*=O). IR (film) υ: 3284, 2985, 2938, 2872, 1737. ESI-HRMS: m/z calcd for $C_{17}H_{23}NO_7SNa$ $[M+Na]^+$ 408.1087; found 408.1122.

3,6-anhydro-2,7-dideoxy-4,5-*O*-(1-methylethylidene)-7-benzosulfonylamino-L-*galacto*-heptonic acid 48

Prepared starting from **47** (500 mg, 1.30 mmol) following procedure described for **21**. Yield: 90% (434 mg). Colorless gum. *Rf;* 0.10 (cH/EA: 1/2). $[\alpha]_D$= +11.3° (c = 0.08, $CHCl_3$). ^{1}H NMR (250 MHz, $CDCl_3$): δ = 1.28 (s, 3H, C(C*H*$_3$)$_2$), 1.37 (s, 3H, C(C*H*$_3$)$_2$), 2.69 (dd, 1H, J_{gem} = 17.0, $J_{2,3}$ = 6.5 Hz, *H*-2), 2.79 (dd, 1H, $J_{2',3}$ = 6.5 Hz, *H*-2'), 3.28 (t, 2H, $J_{7,NH}$ = 6.5, $J_{6,7}$ = 6.5 Hz, *H*-7), 3.66 (td, 1H, $J_{5,6}$ = 3.5 Hz, *H*-6), 3.88 (td, 1H, $J_{3,4}$ = 3.5 Hz, *H*-3), 4.65 (dd, 1H, $J_{4,5}$ = 6.0 Hz, *H*-4), 4.71 (dd, 1H, *H*-5), 5.02 (t, 1H, N*H*), 7.48-7.61 (m, 3H, *H*-Ar), 7.85-7.89 (m, 2H, *H*-Ar). ^{13}C NMR (62.9 MHz, $CDCl_3$): δ = 24.7 (*C*H$_3$), 25.6 (*C*H$_3$), 33.3 (*C*-2), 42.1 (*C*-7), 77.2 (*C*-3), 79.3 (*C*-6), 80.8, 81.1 (*C*-4, *C*-5), 112.9 (*C*(CH$_3$)$_2$), 127.0 (2 *C*-Ar), 129.1 (2 *C*-Ar), 132.7 (*C*-Ar), 139.9 (*C*-Ar), 175.7 (*C*=O). IR (film) υ: 3270, 2985, 2928, 2857, 1716. ESI-HRMS: m/z calcd for $C_{16}H_{21}NO_7SNa$ $[M+Na]^+$ 394.0931; found 394.0953.

***N*$^\alpha$-[2-*C*-[2,3-*O*-(1-methylethylidene)-5-benzosulfonylamino-α-D-galactofuranosyl]carbonymethyl)]-L-nitro-arginine methyl ester 49**

Prepared starting from **48** (400 mg, 1.08 mmol) following procedure described for **39**. Yield: 84% (531 mg). White foam. *Rf;* 0.4 (EA/MeOH: 9/1). $[\alpha]_D$= +5.1° (c = 0.4, $CHCl_3$). ^{1}H NMR (400 MHz, $CDCl_3$): δ = 1.25 (s, 3H, C(C*H*$_3$)$_2$), 1.38 (s, 3H, C(C*H*$_3$)$_2$), 1.65-1.76 (m, 3H, C*H*$_2$ *arg*), 1.93 (m, 1H, C*H*$_2$ *arg*), 2.54 (dd, 1H, J_{gem} = 15.0, $J_{2,3}$ = 3.5 Hz, *H*-2), 2.71 (dd, 1H, $J_{2',3}$ = 9.0 Hz, *H*-2'), 3.22 (dd, 1H, J_{gem} = 13.0, $J_{6,7}$ = 8.0 Hz, *H*-7), 3.30-3.37 (m, 2H, *H*-7', C*H*$_2$ *arg*), 3.56 (m, 1H, C*H*$_2$ *arg*), 3.68 (m, 1H, *H*-6), 3.79-3.92 (m, 4H, *H*-3, OC*H*$_3$), 4.56-4.65 (m, 3H, *H*-α, *H*-4, *H*-5), 5.77 (br s, 1H, N*H*), 7.13 (d, 1H, *J* = 7.0 Hz, N*H*), 7.52-7.62 (m, 5H, 3 *H*-Ar, 2 N*H*), 7.90 (d, 2H, *J* = 7.5 Hz, *H*-Ar), 8.66 (br s, 1H, N*H*). ^{13}C NMR (100 MHz, $CDCl_3$): δ = 24.5 (*C*H$_2$ *arg*, *C*H$_3$), 25.7 (*C*H$_3$), 30.5 (*C*H$_2$ *arg*), 36.1 (*C*-2), 40.5 (*C*H$_2$ *arg*), 42.3 (*C*-7), 51.1 (*C*-α), 53.1 (O*C*H$_3$), 77.7 (*C*-3), 79.9 (*C*-6), 80.8, 81.6 (*C*-4, *C*-5), 112.9 (*C*(CH$_3$)$_2$), 127.0 (2 *C*-Ar), 129.2 (2 *C*-Ar), 132.7 (*C*-Ar), 139.9 (*C*-Ar), 159.3 (*C*=N), 172.1 (*C*=O), 173.0 (*C*=O). IR (film) υ: 3360, 2933, 2867, 1735, 1633, 1539. ESI-HRMS: m/z calcd for $C_{23}H_{34}N_6O_{10}SNa$ $[M+Na]^+$ 609.1949; found 609.1969.

N^{α}-[2-*C*-[2,3-*O*-(1-methylethylidene)-5-benzosulfonylamino-α-D-galactofuranosyl]carbonymethyl)]-L-arginine methyl ester 50

Prepared starting from **49** (500 mg, 0.85 mmol) following procedure described for **40**. Yield: 82% (367 mg). White solid. *Rf;* 0.24 (H_2O/MeOH: 1/1). HPLC (70/30): t_R = 6.049 min. $[\alpha]_D$= +15.0° (c = 0.2, H_2O). ^{1}H NMR (400 MHz, D_2O): δ = 1.22 (s, 3H, C(C*H*$_3$)$_2$), 1.33 (s, 3H, C(C*H*$_3$)$_2$), 1.49-1.56 (m, 2H, C*H*$_2$ *arg*), 1.64 (m, 1H, C*H*$_2$ *arg*), 1.77 (m, 1H, C*H*$_2$ *arg*), 2.53-2.63 (m, 2H, *H*-2), 3.07-3.11 (m, 3H, *H*-7, 2 C*H*$_2$ *arg*), 3.18 (dd, 1H, J_{gem} = 13.5, $J_{6,7'}$ = 4.5 Hz, *H*-7'), 3.60 (m, 1H, *H*-6), 3.85 (td, 1H, $J_{3,4}$ = 2.5 Hz, *H*-3), 4.12 (dd, 1H, $J_{H\text{-}\alpha,A1}$ = 8.0 Hz, $J_{H\text{-}\alpha,A1'}$ = 5.0 Hz, *H*-α), 4.67-4.69 (m, 2H, *H*-4, *H*-5), 7.53-7.57 (m, 2H, *H*-Ar), 7.64 (m, 1H, *H*-Ar), 7.79-7.82 (m, 2H, *H*-Ar). ^{13}C NMR (100 MHz, D_2O): δ = 23.6 (*C*H$_3$), 24.4 (*C*H$_2$ *arg*), 24.7 (*C*H$_3$), 28.8 (*C*H$_2$ *arg*), 34.7 (*C*-2), 40.6 (*C*H$_2$ *arg*), 41.3 (*C*-7), 54.8 (*C*-α), 77.7 (*C*-3), 79.1 (*C*-6), 80.3 (*C*-5), 81.1 (*C*-4), 112.8 (*C*(CH$_3$)$_2$), 126.7 (2 *C*-Ar), 129.5 (2 *C*-Ar), 133.6 (*C*-Ar), 137.9 (*C*-Ar), 156.7 (*C*=N), 171.9 (*C*=O), 178.4 (*C*=O). IR (pellets) υ: 3360, 3180, 2990, 2943, 2876, 1645, 1586. ESI-HRMS: m/z calcd for $C_{22}H_{33}N_5O_8SNa$ $[M+Na]^+$ 550.1942; found 550.1956.

3,6-Anhydro-2-deoxy-4,5-*O*-(1-methylethylidene)-7-(4-methylbenzenesulfonate)-D-*altro*-heptonic acid methyl ester 51

Prepared starting from **19** (1.0 g, 4.07 mmol) following procedure described for **8**. Yield: 98% (1.59 g). Colorless oil. *Rf;* 0.71 (cH/EA: 1/1). $[\alpha]_D$= +11.0° (c = 0.1, $CHCl_3$). ^{1}H NMR (250 MHz, $CDCl_3$): δ = 1.31 (s, 3H, C(C*H*$_3$)$_2$), 1.45 (s, 3H, C(C*H*$_3$)$_2$), 2.46 (s, 3H, Ph-C*H*$_3$), 2.63 (dd, 1H, J_{gem} = 16.5, $J_{2,3}$ = 7.0 Hz, *H*-2), 2.74 (dd, 1H, $J_{2',3}$ = 7.0 Hz, *H*-2'), 3.70 (s, 3H, OC*H*$_3$), 4.00 (dd, 1H, J_{gem} = 10.5, $J_{6,7}$ = 5.0 Hz, *H*-7), 4.08 (dd, 1H, $J_{6,7'}$ = 4.5 Hz, *H*-7'), 4.18 (m, 1H, *H*-6), 4.29 (td, 1H, $J_{3,4}$ = 4.0 Hz, *H*-3), 4.71 (dd, 1H, $J_{4,5}$ = 6.0, $J_{5,6}$ = 0.5 Hz, *H*-5), 4.77 (dd, 1H, *H*-4), 7.36 (d, 2H, *J* = 8.0 Hz, *H*-Ar), 7.80 (d, 2H, *H*-Ar). ^{13}C NMR (62.9 MHz, $CDCl_3$): δ = 21.8 (Ph-*C*H$_3$), 25.0 (*C*H$_3$), 26.3 (*C*H$_3$), 34.5 (*C*-2), 51.9 (O*C*H$_3$), 69.6 (*C*-7), 78.4, 81.4, 82.7 (*C*-3, *C*-4, *C*-5, *C*-6), 113.1 (*C*(CH$_3$)$_2$), 128.2 (2 *C*-Ar), 130.2 (2 *C*-Ar), 132.6 (*C*-Ar), 145.3 (*C*-Ar), 171.4 (*C*=O). IR (film) υ: 2985, 2943, 1737, 1596. ESI-HRMS: m/z calcd for $C_{18}H_{24}O_8SNa$ $[M+Na]^+$ 423.1084; found 423.1087.

3,6-anhydro-2,7-dideoxy-4,5-*O*-(1-methylethylidene)-7-azido-D-*altro*-heptonic acid methyl ester 52

Prepared starting from **51** (1.4 g, 3.5 mmol) following procedure described for **30**. Yield: 97% (920 mg). Colorless oil. *Rf;* 0.50 (cH/EA: 2/1). $[\alpha]_D$= +18.5° (c = 0.2, $CHCl_3$). ^{1}H NMR (250 MHz, $CDCl_3$): δ = 1.33 (s, 3H, C(C*H*$_3$)$_2$), 1.49 (s, 3H, C(C*H*$_3$)$_2$), 2.73 (dd, 1H, J_{gem} = 17.0, $J_{2,3}$ = 7.0 Hz, *H*-2), 2.81 (dd, 1H, $J_{2',3}$ = 7.0 Hz, *H*-2'), 3.28 (dd, 1H, J_{gem} = 13.0, $J_{6,7}$ = 5.0 Hz, *H*-7), 3.43 (dd, 1H, $J_{6,7'}$ = 6.5 Hz, *H*-7'), 3.71 (s, 3H, OC*H*$_3$), 4.21 (m, 1H, *H*-6), 4.41 (td, 1H, $J_{3,4}$ = 4.0 Hz, *H*-3), 4.64 (dd, 1H, $J_{4,5}$ = 6.0, $J_{5,6}$ = 0.5 Hz, *H*-5), 4.80 (dd, 1H, *H*-4). ^{13}C NMR (62.9 MHz, $CDCl_3$): δ = 25.2 (*C*H$_3$), 26.4 (*C*H$_3$), 34.5 (*C*-2), 51.8, 52.0 (*C*-7, O*C*H$_3$), 77.6 (*C*-3), 81.5, 82.9, 83.4 (*C*-4, *C*-5, *C*-6), 113.2 (*C*(CH$_3$)$_2$), 171.5 (*C*=O). IR (film) υ: 2990, 2938, 2099, 1737. ESI-HRMS: m/z calcd for $C_{11}H_{17}N_3O_5Na$ $[M+Na]^+$ 294.1060; found 294.1073.

3,6-anhydro-2,7-dideoxy-4,5-*O*-(1-methylethylidene)-7-benzosulfonylamino-D-*altro*-heptonic acid methyl ester 53

Prepared starting from **52** (200 mg, 0.74 mmol) following procedure described for **47**. Yield: 83% (235 mg). Colorless oil. *Rf;* 0.29 (cH/EA: 7/3). $[\alpha]_D$= +26.2° (c = 0.14, $CHCl_3$). ^{1}H NMR (250 MHz, $CDCl_3$): δ = 1.29 (s, 3H, C(C*H*$_3$)$_2$), 1.44 (s, 3H, C(C*H*$_3$)$_2$), 2.64 (dd, 1H, J_{gem} = 16.0, $J_{2,3}$ = 7.0 Hz, *H*-2), 2.74 (dd, 1H, $J_{2',3}$ = 7.0 Hz, *H*-2'), 2.89 (ddd, 1H, J_{gem} = 13.0, $J_{6,7}$ = 8.5, $J_{7,NH}$ = 4.0 Hz, *H*-7), 3.07 (ddd, 1H, $J_{6,7'}$ = 5.0, $J_{7',NH}$ = 8.0 Hz, *H*-7'), 3.71 (s, 3H, OC*H*$_3$), 4.05 (ddd, 1H, $J_{5,6}$ = 1.0 Hz, *H*-6), 4.12 (td, 1H, $J_{3,4}$ = 4.0 Hz, *H*-3), 4.53 (dd, 1H, $J_{4,5}$ = 6.0 Hz, *H*-5), 4.69 (dd, 1H, *H*-4), 4.78 (dd, 1H, N*H*), 7.49-7.63 (m, 3H, *H*-Ar), 7.85-7.89 (m, 2H, *H*-Ar).

^{13}C NMR (62.9 MHz, $CDCl_3$): δ = 25.1 (*C*H_3), 26.3 (*C*H_3), 34.1 (*C*-2), 42.8 (*C*-7), 52.0 (O*C*H_3), 76.5 (*C*-3), 81.1, 81.9, 83.1 (*C*-4, *C*-5, *C*-6), 113.3 (*C*$(CH_3)_2$), 127.2 (2 *C*-Ar), 129.4 (2 *C*-Ar), 133.0 (*C*-Ar), 139.7 (*C*-Ar), 171.5 (*C*=O). IR (film) υ: 3279, 2990, 2933, 1735. ESI-HRMS: m/z calcd for $C_{17}H_{23}NO_7SNa$ $[M+Na]^+$ 408.1087; found 408.1090.

3,6-anhydro-2,7-dideoxy-4,5-*O*-(1-methylethylidene)-7-benzosulfonylamino-D-*altro*-heptonic acid 54

Prepared starting from **53** (200 mg, 0.52 mmol) following procedure described for **21**. Yield: 96% (185 mg). Colorless oil. *Rf;* 0.09 (cH/EA: 1/2). $[\alpha]_D$= +23.7° (c = 0.2, $CHCl_3$). ^{1}H NMR (250 MHz, $CDCl_3$): δ = 1.30 (s, 3H, C(C*H*$_3$)$_2$), 1.45 (s, 3H, C(C*H*$_3$)$_2$), 2.69 (dd, 1H, J_{gem} = 17.0, $J_{2,3}$ = 6.0 Hz, *H*-2), 2.78 (dd, 1H, $J_{2',3}$ = 6.0 Hz, *H*-2'), 2.91 (ddd, 1H, J_{gem} = 13.0, $J_{6,7}$ = 8.0, $J_{7,NH}$ = 4.0 Hz, *H*-7), 3.08 (ddd, 1H, $J_{6,7'}$ = 5.0, $J_{7',NH}$ = 8.0 Hz, *H*-7'), 4.09 (ddd, 1H, $J_{5,6}$ = 1.0 Hz, *H*-6), 4.14 (td, 1H, $J_{3,4}$ = 4.0 Hz, *H*-3), 4.56 (dd, 1H, $J_{4,5}$ = 6.0 Hz, *H*-5), 4.71 (dd, 1H, *H*-4), 5.12 (dd, 1H, N*H*), 7.48-7.62 (m, 3H, *H*-Ar), 7.84-7.89 (m, 2H, *H*-Ar). ^{13}C NMR (62.9 MHz, $CDCl_3$): δ = 25.1 (*C*H_3), 26.3 (*C*H_3), 34.1 (*C*-2), 42.8 (*C*-7), 76.5 (*C*-3), 81.1, 82.1, 83.1 (*C*-4, *C*-5, *C*-6), 113.4 (*C*$(CH_3)_2$), 127.2 (2 *C*-Ar), 129.4 (2 *C*-Ar), 133.0 (*C*-Ar), 139.6 (*C*-Ar), 175.4 (*C*=O). IR (film) υ: 3265, 2985, 2928, 1716. ESI-HRMS: m/z calcd for $C_{16}H_{21}NO_7SNa$ $[M+Na]^+$ 394.0931; found 394.0929.

N^{α}-[2-*C*-[2,3-*O*-(1-methylethylidene)-5-benzosulfonylamino-α-D-ribofuranosyl]carbonymethyl]-L-nitro-arginine methyl ester 55

Prepared starting from **54** (170 mg, 0.46 mmol) following procedure described for **39**. Yield: 71% (191 mg). White solid. *Rf;* 0.37 (EA/MeOH: 9/1). $[\alpha]_D$= +7.0° (c = 0.5, $CHCl_3$). ^{1}H NMR (250 MHz, $CDCl_3$): δ = 1.27 (s, 3H, C(C*H*$_3$)$_2$), 1.44 (s, 3H, C(C*H*$_3$)$_2$), 1.63-1.83 (m, 3H, C*H*$_2$ *arg*), 1.93 (m, 1H, C*H*$_2$ *arg*), 2.54 (dd, 1H, J_{gem} = 15.5, $J_{2,3}$ = 3.0 Hz, *H*-2), 2.75 (dd, 1H, $J_{2',3}$ = 9.5 Hz, *H*-2'), 2.89 (m, 1H, *H*-7), 3.06 (ddd, 1H, J_{gem} = 13.5, *J* = 8.0, *J* = 4.5 Hz, *H*-7'), 3.31 (m, 1H, C*H*$_2$ *arg*), 3.47 (m, 1H, C*H*$_2$ *arg*), 3.78 (s, 3H, OC*H*$_3$), 4.12 (m, 1H, *H*-6), 4.19 (m, 1H, *H*-3), 4.49 (d, 1H, $J_{4,5}$ = 6.0 Hz, *H*-5), 4.57-4.65 (m, 2H, *H*-α, *H*-4), 6.17 (br s, 1H, N*H*), 7.26 (m, 1H, N*H*), 7.47-7.62 (m, 5H, 3 *H*-Ar, 2 N*H*), 7.83-7.88 (m, 2H, *H*-Ar), 8.59 (br s, 1H, N*H*). ^{13}C NMR (62.9 MHz, $CDCl_3$): δ = 25.0 (*C*H_2 *arg* , *C*H_3), 26.4 (*C*H_3), 30.2 (*C*H_2 *arg*), 36.8 (*C*-2), 40.8 (*C*H_2 *arg*), 42.8 (*C*-7), 51.5 (*C*-α), 53.2 (O*C*H_3), 76.6 (*C*-3), 81.6, 83.1, 83.3 (*C*-4, *C*-5, *C*-6), 113.3 (*C*$(CH_3)_2$), 127.1 (2 *C*-Ar), 129.5 (2 *C*-Ar), 133.0 (*C*-Ar), 140.1 (*C*-Ar), 159.6 (*C*=N), 172.2 (*C*=O), 173.5 (*C*=O). IR (film) υ: 3303, 2990, 2933, 2876, 1737, 1636, 1598, 1537. ESI-HRMS: m/z calcd for $C_{23}H_{34}N_6O_{10}SNa$ $[M+Na]^+$ 609.1949; found 609.1940.

N^{α}-[2-*C*-[2,3-*O*-(1-methylethylidene)-5-benzosulfonylamino-α-D-ribofuranosyl]carbonylmethyl)]-L-arginine methyl ester 56

Prepared starting from **55** (150 mg, 0.26 mmol) following procedure described for **40**. Yield: 70% (94 mg). White solid. *Rf;* 0.22 (H_2O/MeOH: 1/1). HPLC (70/30): t_R = 5.526 min. $[\alpha]_D$= +17.1° (c = 0.1, H_2O). ^{1}H NMR (250 MHz, D_2O): δ = 1.37 (s, 3H, C(C*H*$_3$)$_2$), 1.52 (s, 3H, C(C*H*$_3$)$_2$), 1.59-1.95 (m, 4H, C*H*$_2$ *arg*), 2.63 (dd, 1H, J_{gem} = 14.5, $J_{2,3}$ = 7.5 Hz, *H*-2), 2.72 (dd, 1H, $J_{2,3'}$ = 6.5 Hz, *H*-2'), 3.06 (dd, 1H, J_{gem} = 14.0, $J_{6,7}$ = 8.0 Hz, *H*-7), 3.14 (dd, 1H, $J_{6,7'}$ = 6.0 Hz, *H*-7'), 3.23 (t, 2H, *J* = 7.0 Hz, C*H*$_2$ *arg*), 4.12 (dd, 1H, *H*-6), 4.20-4.27 (m, 2H, *H*-3, *H*-α), 4.74 (d, 1H, $J_{4,5}$ = 6.0 Hz, *H*-5), 4.84 (dd, 1H, $J_{3,4}$ = 4.0 Hz, *H*-4), 7.66-7.80 (m, 3H, *H*-Ar), 7.91-7.95 (m, 2H, *H*-Ar). ^{13}C NMR (62.9 MHz, D_2O): δ = 23.6, 24.4, 25.0 (2 *C*H_3, *C*H_2 *arg*), 28.8 (*C*H_2 *arg*), 35.2 (*C*-2), 40.7 (*C*H_2 *arg*), 41.8 (*C*-7), 54.6 (*C*-α), 76.6 (*C*-3), 80.7, 82.1, 82.4 (*C*-4, *C*-5, *C*-6), 113.1 (*C*$(CH_3)_2$), 126.6 (2 *C*-Ar), 129.6 (2 *C*-Ar), 133.6 (*C*-Ar), 138.3 (*C*-Ar), 156.7 (*C*=N), 171.7 (*C*=O), 178.5 (*C*=O). IR (pellets) υ: 3331, 3134, 2988, 2928, 2860, 1632, 1575, 1446. ESI-HRMS: m/z calcd for $C_{22}H_{33}N_5O_8SNa$ $[M+Na]^+$ 550.1942; found 550.1957.

3,6-Anhydro-2,7-dideoxy-4,5-*O*-(1-methylethylidene)-7-[[(4-bromomethyl)benzo]sulfonylamino]-D-*altro*-heptonic acid methyl ester 57

To a solution of **52** (165 mg, 0.6 mmol, 1.0 eq.) in MeOH (10 mL) was added Pd/C (10%) (17 mg, 10% w/w). After stirring under H_2 atmosphere (15 psi) until completion of the reaction, the mixture was filtered through a pad of celite and the filtrate was concentrated *in vacuo*. The crude amine was solubilized in CH_2Cl_2 (1.5 mL) under argon and pyridine (165 µL, 2.1 mmol, 3.5 eq.) and (4-bromomethylbenzyl)sulfonyl chloride (178 mg, 0.66 mmol, 1.1 eq.) were added dropwise. After stirring at room temperature for 30 min, the mixture was diluted with CH_2Cl_2 (10 mL) and washed with 1N HCl (2x5 mL). The organic layer was dried over $MgSO_4$, filtered and the solvent was evaporated under reduced pressure. The compound was purified by flash chromatography (silica gel, cyH/EA) to provide **57** (174 mg, 61%). Colorless oil. *Rf;* 0.43 (cH/EA: 1/1). $[\alpha]_D$= +18.5° (c = 0.2, $CHCl_3$). ^{1}H NMR (250 MHz, $CDCl_3$): δ = 1.29 (s, 3H, C(C*H*$_3$)$_2$), 1.45 (s, 3H, C(C*H*$_3$)$_2$), 2.64 (dd, 1H, J_{gem} = 15.5, $J_{2,3}$ = 6.5 Hz, *H*-2), 2.74 (dd, 1H, $J_{2',3}$ = 6.5 Hz, *H*-2'), 2.90 (ddd, 1H, J_{gem} = 13.0, $J_{6,7}$ = 8.5, $J_{7,NH}$ = 4.0 Hz, *H*-7), 3.08 (ddd, 1H, $J_{6,7'}$ = 5.0, $J_{7',NH}$ = 8.0 Hz, *H*-7'), 3.71 (s, 3H, OC*H*$_3$), 4.06 (ddd, 1H, $J_{5,6}$ = 1.0 Hz, *H*-6), 4.12 (td, 1H, $J_{3,4}$ = 4.0 Hz, *H*-3), 4.53 (dd, 1H, $J_{4,5}$ = 6.0 Hz, *H*-5), 4.62 (s, 2H, C*H*$_2$-Br), 4.70 (dd, 1H, *H*-4), 4.81 (dd, 1H, N*H*), 7.52-7.58 (m, 2H, *H*-Ar), 7.84-7.89 (m, 2H, *H*-Ar). ^{13}C NMR (62.9 MHz, $CDCl_3$): δ = 24.9 (*C*H$_3$), 26.1 (*C*H$_3$), 33.9 (*C*-2), 42.6 (*C*-7), 44.8 (*C*H$_2$-Br), 51.8 (O*C*H$_3$), 76.3 (*C*-3), 80.9, 81.7, 82.9 (*C*-4, *C*-5, *C*-6), 113.2 (*C*(CH$_3$)$_2$), 127.5 (2 *C*-Ar), 129.2 (2 *C*-Ar), 139.5 (*C*-Ar), 142.4 (*C*-Ar), 171.3 (*C*=O). IR (film) υ: 3274, 2985, 2924, 2848, 1735, 1440. ESI-HRMS: m/z calcd for $C_{18}H_{24}BrNO_7SNa$ $[M+Na]^+$ 500.0349; found 500.0317.

3,6-Anhydro-2,7-dideoxy-4,5-*O*-(1-methylethylidene)-7-[[(4-azidomethyl)benzo]sulfonylamino]-D-*altro*-heptonic acid methyl ester 58

To a stirred solution of compound **57** (120 mg, 0.25 mmol, 1 eq.) in DMF (2 mL) was added sodium azide (97 mg, 1.5 mmol, 6 eq.) and the mixture was stirred for 15 hours at 60°C. The solvent was removed in vacuo and the residue was diluted with CH_2Cl_2 (10 mL), washed with H_2O (5 mL) and brine (5 mL). The organic layer was dried over $MgSO_4$, filtered and the solvent was evaporated under reduced pressure. The compound was purified by flash chromatography (silica gel, cyH/EA) to provide **58** (110 mg, quantitative yield). White solid. *Rf;* 0.21 (cH/EA: 7/3). $[\alpha]_D$= +21.7° (c = 0.25, $CHCl_3$). ^{1}H NMR (250 MHz, $CDCl_3$): δ = 1.29 (s, 3H, C(C*H*$_3$)$_2$), 1.45 (s, 3H, C(C*H*$_3$)$_2$), 2.65 (dd, 1H, J_{gem} = 16.5, $J_{2,3}$ = 7.0 Hz, *H*-2), 2.73 (dd, 1H, $J_{2',3}$ = 6.5 Hz, *H*-2'), 2.88 (ddd, 1H, J_{gem} = 13.0, $J_{6,7}$ = 9.0, $J_{7,NH}$ = 3.5 Hz, *H*-7), 3.08 (ddd, 1H, $J_{6,7'}$ = 5.0, $J_{7',NH}$ = 8.0 Hz, *H*-7'), 3.71 (s, 3H, OC*H*$_3$), 4.03-4.14 (m, 2H, *H*-6, *H*-3), 4.46 (s, 2H, C*H*$_2$-N$_3$), 4.53 (dd, 1H, $J_{4,5}$ = 6.0, $J_{5,6}$ = 1.5 Hz, *H*-5), 4.69 (dd, 1H, $J_{3,4}$ = 4.0 Hz, *H*-4), 4.84 (dd, 1H, N*H*), 7.49 (d, 2H, *J* = 8.0 Hz, 2 *H*-Ar), 7.89 (d, 2H, 2 *H*-Ar). ^{13}C NMR (62.9 MHz, $CDCl_3$): δ = 25.1 (*C*H$_3$), 26.3 (*C*H$_3$), 34.0 (*C*-2), 42.7 (*C*-7), 52.0 (O*C*H$_3$), 54.1 (*C*H$_2$-N$_3$), 76.5 (*C*-3), 81.1, 81.9, 83.1 (*C*-4, *C*-5, *C*-6), 113.4 (*C*(CH$_3$)$_2$), 127.8 (2 *C*-Ar), 128.8 (2 *C*-Ar), 139.5 (*C*-Ar), 140.9 (*C*-Ar), 171.5 (*C*=O). IR (pellets) υ: 3274, 2990, 2933, 2104, 1735. ESI-HRMS: m/z calcd for $C_{18}H_{24}N_4O_7SNa$ $[M+Na]^+$ 463.1258; found 463.1207.

3,6-Anhydro-2,7-dideoxy-4,5-*O*-(1-methylethylidene)-7-[[(4-azidomethyl)benzo]sulfonylamino]-D-*altro*-heptonic acid 59

Prepared starting from **58** (100 mg, 0.23 mmol) following procedure described for **30**. Yield: quantitative yield (98 mg). Colorless oil. *Rf;* 0.21 (cH/EA: 1/3). $[\alpha]_D$= +15.7° (c = 0.3, $CHCl_3$). ^{1}H NMR (250 MHz, $CDCl_3$): δ = 1.29 (s, 3H, C(C*H*$_3$)$_2$), 1.45 (s, 3H, C(C*H*$_3$)$_2$), 2.63-2.80 (m, 2H, *2 H*-2), 2.91 (m, 1H, *H*-7), 3.07 (m, 1H, *H*-7'), 4.04-4.17 (m, 2H, *H*-6, *H*-3), 4.45 (s, 2H, C*H*$_2$-N$_3$), 4.56 (d, 1H, $J_{4,5}$ = 6.0 Hz, *H*-5), 4.70 (dd, 1H, $J_{3,4}$ = 4.0 Hz, *H*-4), 5.39 (dd, 1H, N*H*), 7.47 (d, 2H, *J* = 8.0 Hz, 2 *H*-Ar), 7.87 (d, 2H, 2 *H*-Ar). ^{13}C NMR (62.9 MHz, $CDCl_3$): δ = 24.9 (*C*H$_3$), 26.1 (*C*H$_3$), 34.1 (*C*-2), 42.6 (*C*-7), 53.9 (*C*H$_2$-Br), 76.4 (*C*-3), 81.0, 82.0, 82.9 (*C*-4, *C*-

5, *C*-6), 113.2 (*C*(CH_3)$_2$), 127.6 (2 *C*-Ar), 128.6 (2 *C*-Ar), 139.3 (*C*-Ar), 140.7 (*C*-Ar), 175.6 (*C*=O). IR (pellets) υ: 3265, 2985, 2924, 2857, 2104, 1716. ESI-HRMS: m/z calcd for $C_{17}H_{22}N_4O_7SNa$ $[M+Na]^+$ 449.1101; found 449.1130.

N^{α}-[2-*C*-[2,3-*O*-(1-methylethylidene)-5-[[(4-azidomethyl)benzo]sulfonylamino]-α-D-ribofuranosyl]carbonymethyl)]]-L-arginine methyl ester 60

To a solution of H-L-Arg-OMe.2HCl (55 mg, 0.21 mmol, 1.1 eq.) and **59** (80 mg, 0.19 mmol, 1 eq.) in DMF (10 mL) were added HATU (108 mg, 0.29 mmol, 1.5 eq.) and DIEA (105 µL, 0.61 mmol, 3.2 eq.) at 0°C under argon atmosphere. The reaction mixture was stirred overnight at room temperature and the solvent was evaporated. This crude product was solubilized in THF (3 mL) and water (1 mL) and LiOH (30 mg, 0.57 mmol, 3 eq.) was added. The mixture was stirred until completion of the reaction monitored by tlc. Amberlite® IR-120 was added until pH = 3-4 and the mixture was filtered off. The solvent were evaporated and the product was purified by column chromatography (C-18 silica gel, H_2O/MeOH) and lyophilized to provide **60** (95 mg, 86%). White solid. *Rf;* 0.18 (H_2O/MeOH: 1/1). $[\alpha]_D$= +31.0° (c = 0.1, MeOH). ^{1}H NMR (250 MHz, MeOH-d_4): δ = 1.29 (s, 3H, C(C*H*$_3$)$_2$), 1.43 (s, 3H, C(C*H*$_3$)$_2$), 1.57-1.70 (m, 2H, C*H*$_2$ *arg*), 1.75 (m, 1H, C*H*$_2$ *arg*), 1.87 (m, 1H, C*H*$_2$ *arg*), 2.53 (dd, 1H, J_{gem} = 15.5, $J_{2,3}$ = 5.5 Hz, *H*-2), 2.63 (dd, 1H, $J_{2',3}$ = 8.0 Hz, *H*-2'), 2.87-3.04 (m, 2H, 2 *H*-7), 3.17-3.23 (m, 2H, C*H*$_2$ *arg*), 4.02 (dd, 1H, $J_{6,7}$ = 8.0, $J_{6,7'}$ = 6.0 Hz, *H*-6), 4.17 (m, 1H, *H*-3), 4.28 (dd, 1H, *J* = 7.5, *J* = 5.5 Hz, *H*-α), 4.50 (s, 2H, C*H*$_2$-N_3), 4.62-4.69 (m, 2H, *H*-4, *H*-5), 7.55 (d app., 2H, *J* = 8.5 Hz, 2 *H*-Ar), 7.90 (d app., 2H, 2 *H*-Ar). ^{13}C NMR (62.9 MHz, MeOH-d_4): δ = 23.9 (*C*H$_3$), 24.8 (*C*H$_2$ *arg*), 25.4 (*C*H$_3$), 30.0 (*C*H$_2$ *arg*), 36.1 (*C*-2), 41.0 (*C*H$_2$ *arg*), 42.6 (*C*-7), 53.6, 54.2 (*C*H$_2$-N_3, *C*-α), 77.0 (*C*-3), 81.6, 83.2, 83.4 (*C*-4, *C*-5, *C*-6), 112.5 (*C*(CH$_3$)$_2$), 127.3 (2 *C*-Ar), 128.7 (2 *C*-Ar), 140.5 (*C*-Ar), 141.1 (*C*-Ar), 157.4 (*C*=N), 171.3 (*C*=O), 177.2 (*C*=O). IR (pellets) υ: 3364, 3184, 2981, 2938, 2104, 1648, 1598. ESI-HRMS: m/z calcd for $C_{23}H_{34}N_8O_8SNa$ $[M+Na]^+$ 605.2113; found 605.2118.

N^{α}-[2-*C*-[2,3-*O*-(1-methylethylidene)-5-[((4-aminomethyl)benzo)sulfonylamino]-α-D-ribofuranosyl]carbonymethyl)]]-L-arginine methyl ester 61

To a solution of **60** (80 mg, 0.14 mmol) in MeOH (5 mL), was added Pd/C (10%) (8 mg, 10% w/w). After stirring under H_2 atmosphere (15 psi) until completion of the reaction, the mixture was filtered through a pad of celite and the filtrate was concentrated *in vacuo*. The crude was purified by column chromatography (C-18 silica gel, H_2O/MeOH) and lyophilized to provide **61** (65 mg, 87%). White solid. *Rf;* 0.38 (H_2O/CH_3CN/TFA: 7/3/0.1%). HPLC (70/30): t_R = 3.681 min. $[\alpha]_D$= +6.9° (c = 0.1, H_2O). ^{1}H NMR (400 MHz, DMSO-d_6): δ = 1.25 (s, 3H, C(C*H*$_3$)$_2$), 1.38 (s, 3H, C(C*H*$_3$)$_2$), 1.45-1.61 (m, 3H, C*H*$_2$ *arg*), 1.73 (m, 1H, C*H*$_2$ *arg*), 2.47-2.53 (m, 2H, 2 *H*-2), 2.68 (m, 1H, *H*-7), 2.82 (m, 1H, *H*-7'), 3.07-3.11 (m, 2H, C*H*$_2$ *arg*), 3.87 (t, 1H, $J_{6,7}$ = 7.0 Hz, *H*-6), 4.10-4.17 (m, 3H, *H*-3, C*H*$_2$-NH_2), 4.20 (td, 1H, *J* = 8.0, *J* = 5.0 Hz, *H*-α), 4.63 (d, 1H, $J_{4,5}$ = 6.0 Hz, *H*-5), 4.66 (dd, 1H, $J_{3,4}$ = 3.5 Hz, *H*-4), 6.99 & 7.20 (br s, 4H, 4 N*H*), 7.59 (t, 1H, *J* = 5.5 Hz, N*H*), 7.67 (d, 2H, *J* = 8.0 Hz, 2 *H*-Ar), 7.84-7.90 (m, 3H, 2 *H*-Ar, N*H*), 8.18 (d, 1H, $J_{NH,\alpha}$ = 8.0 Hz, N*H*), 8.28 (br s, 3H, C*H*$_2$-NH_3^+). ^{13}C NMR (100 MHz, DMSO-d_6): δ = 25.2 (*C*H$_3$), 25.5 (*C*H$_2$ *arg*), 26.6 (*C*H$_3$), 28.8 (*C*H$_2$ *arg*), 35.3 (*C*-2), 40.7 (*C*H$_2$ *arg*), 42.2 (*C*-7), 42.9 (*C*H$_2$-NH_2), 51.8 (*C*-α), 77.0 (*C*-3), 81.3 (*C*-4), 82.1 (*C*-6), 83.0 (*C*-5), 111.8 (*C*(CH$_3$)$_2$), 127.3 (2 *C*-Ar), 130.1 (2 *C*-Ar), 139.0 (*C*-Ar), 140.7 (*C*-Ar), 157.1 (*C*=N), 158.5 (TFA), 170.0 (*C*=O amide), 173.9 (*C*=O acid). IR (pellets) υ: 3393, 3189, 2990, 2928, 1676, 1546. ESI-HRMS: m/z calcd for $C_{23}H_{37}N_6O_8S$ $[M+Na]^+$ 557.2388; found 557.2399.

3,6-Anhydro-2,7-dideoxy-4,5-*O*-(1-methylethylidene)-7-[((4-methoxycarbonylmethyl)benzo)sulfonylamino]-D-*altro*-heptonic acid 62

To a solution of **52** (192 mg, 0.71 mmol) in THF (5 mL) and water (2 mL) was added LiOH (51 mg, 2.13 mmol, 3 eq.) and the mixture was stirred until completion of the reaction monitored by tlc. THF was removed under reduced pressure, CH_2Cl_2 (20 mL) was added and the pH was adjusted to 2-3 with aqueous 1N HCl. The organic layer was washed with brine and dried over $MgSO_4$, filtered and concentrated under reduced pressure. This carboxylic acid was solubilized in MeOH (10 mL) and Pd/C (10%) (20 mg, 10% w/w) was added. After stirring under H_2 atmosphere (15 psi) until completion of the reaction, the mixture was filtered through a pad of celite and the filtrate was concentrated *in vacuo*. The crude amine was solubilized in pyridine (1 mL) and this solution was added dropwise under argon atmosphere to a stirred solution of methyl 2-[4-(chlorosulfonyl)phenyl]acetate (212 mg, 0.85 mmol, 1.2 eq.) in pyridine (2 mL). After stirring at room temperature until completion of the reaction, the solvent was removed under reduced pressure. The residue was diluted with CH_2Cl_2 (15 mL) and the pH was adjusted to 3-4 with 1N HCl. The layers were separated and the organic layer was washed with H_2O (2x5 mL). The organic layer was dried over $MgSO_4$, filtered and the solvent was evaporated under reduced pressure. The compound was purified by flash chromatography (silica gel, cyH/EA) to provide **62** (95 mg, 30%). Colorless oil. *Rf;* 0.48 (EA). $[\alpha]_D$= +13.1° (c = 0.1, $CHCl_3$). ^{1}H NMR (250 MHz, $CDCl_3$): δ = 1.30 (s, 3H, C(C*H*$_3$)$_2$), 1.45 (s, 3H, C(C*H*$_3$)$_2$), 2.67 (dd, 1H, J_{gem} = 17.0, $J_{2,3}$ = 7.0 Hz, *H*-2), 2.77 (dd, 1H, $J_{2',3}$ = 6.0 Hz, *H*-2'), 2.91 (m, 1H, *H*-7), 3.09 (m, 1H, *H*-7'), 3.70 (s, 2H, C*H*$_2$-CO$_2$CH$_3$), 3.71 (s, 3H, CO$_2$C*H*$_3$), 4.01-4.18 (m, 2H, *H*-6, *H*-3), 4.57 (dd, 1H, $J_{4,5}$ = 6.0, $J_{5,6}$ = 1.0 Hz, *H*-5), 4.71 (dd, 1H, $J_{3,4}$ = 4.0 Hz, *H*-4), 5.25 (dd, 1H, N*H*), 7.43 (d, 2H, *J* = 8.0 Hz, *H*-Ar), 7.81 (d, 2H, *H*-Ar) , 9.53 (br s, 1H, COO*H*). ^{13}C NMR (62.9 MHz, $CDCl_3$): δ = 24.9 (*C*H$_3$), 26.1 (*C*H$_3$), 34.0 (*C*-2), 40.8 (*C*H$_2$-CO$_2$CH$_3$), 42.7 (*C*-7), 52.3 (CO$_2$*C*H$_3$), 76.4 (*C*-3), 80.9, 82.0, 83.0 (*C*-4, *C*-5, *C*-6), 113.2 (*C*(CH$_3$)$_2$), 127.3 (2 *C*-Ar), 130.2 (2 *C*-Ar), 138.4 (*C*-Ar), 139.1 (*C*-Ar), 171.1 (*C*=O), 175.3 (*C*=O). IR (film) υ: 3270, 2985, 2938, 1730, 1714. ESI-HRMS: m/z calcd for $C_{19}H_{25}NO_9SNa$ $[M+Na]^+$ 466.1142; found 466.1190.

N^{α}-[2-*C*-[2,3-*O*-(1-methylethylidene)-5-[((4-carboxymethyl)benzo)sulfonylamino]-α-D-ribofuranosyl]carbonylmethyl)]]-L-arginine methyl ester 63

Prepared starting from **62** (71mg, 0.16 mmol) following procedure described for **60**. Yield: 54% (50 mg). Colorless oil. *Rf;* 0.75 (H_2O/CH_3CN/TFA: 7/3/0.1%). HPLC (70/30): t_R = 4.383 min. $[\alpha]_D$= +5.0° (c = 0.2, H_2O). ^{1}H NMR (250 MHz, DMSO-d$_6$): δ = 1.22 (s, 3H, C(C*H*$_3$)$_2$), 1.36 (s, 3H, C(C*H*$_3$)$_2$), 1.43-1.62 (m, 3H, C*H*$_2$ *arg*), 1.70 (m, 1H, C*H*$_2$ *arg*), 2.64-2.93 (m, 4H, 2 *H*-2, 2 *H*-7), 3.03-3.12 (m, 2H, C*H*$_2$ *arg*), 3.68 (s, 2H, C*H*$_2$-COOH), 3.84 (t, 1H, $J_{6,7}$ = 7.0, $J_{6,7'}$ = 7.0 Hz, *H*-6), 4.08-4.22 (m, 2H, *H*-3, *H*-α), 4.56-4.64 (m, 2H, *H*-4, *H*-5), 7.08 (br s, 2H, N*H*), 7.46 (d, 2H, *J* = 8.0 Hz, 2 *H*-Ar), 7.73 (d, 2H, *J* = 8.0 Hz, 2 *H*-Ar), 8.12 (m, 1H, N*H*), 12.52 (br s, 2H, 2 COO*H*). ^{13}C NMR (62.9 MHz, DMSO-d$_6$): δ = 25.5 (*C*H$_3$), 25.7 (*C*H$_2$ *arg*), 26.8 (*C*H$_3$), 29.1 (*C*H$_2$ *arg*), 35.6 (*C*-2), 40.8 (*C*H$_2$ *arg*), 41.2 (*C*-7), 43.2 (*C*H$_2$-COOH), 52.0 (*C*-α), 77.2 (*C*-3), 81.5, 82.3, 83.3 (*C*-4, *C*-5, *C*-6), 112.0 (*C*(CH$_3$)$_2$), 127.1 (2 *C*-Ar), 131.0 (2 *C*-Ar), 139.4 (*C*-Ar), 140.6 (*C*-Ar), 157.4 (*C*=N), 159.1 (TFA), 170.2, 172.8, 174.1 (3 *C*=O). IR (pellets) υ: 3374, 3213, 2985, 2938, 1718, 1664, 1648, 1544. ESI-HRMS: m/z calcd for $C_{24}H_{35}N_5O_{10}SNa$ $[M+Na]^+$ 608.2026; found 608.1997.

3,6-Anhydro-2,7-deoxy-4,5-*O*-(1-methylethylidene)-7-[((4-iodo)benzo)sulfonylamino]-D-*altro*-heptonic acid 64

Prepared starting from **52** (100 mg, 0.37 mmol) and (4-iodo-benzo)sulfonyl chloride (135 mg, 0.44 mmol, 1.2 eq.) following procedure described for **47**. Yield: 82% (155 mg). Colorless oil. *Rf;* 0.25 (cH/EA: 7/3). $[\alpha]_D$= +18.5° (c = 0.8, $CHCl_3$). ^{1}H NMR (250 MHz, $CDCl_3$): δ = 1.32 (s, 3H, C(C*H*$_3$)$_2$), 1.47 (s, 3H, C(C*H*$_3$)$_2$), 2.67 (dd, 1H, J_{gem} = 16.0, $J_{2,3}$ = 7.0 Hz, *H*-2), 2.76

(dd, 1H, $J_{2',3}$ = 7.0 Hz, *H*-2'), 2.91 (ddd, 1H, J_{gem} = 13.0, $J_{6,7}$ = 9.0, $J_{7,NH}$ = 4.0 Hz, *H*-7), 3.09 (ddd, 1H, $J_{6,7'}$ = 5.0, $J_{7',NH}$ = 8.0 Hz, *H*-7'), 3.73 (s, 3H, OCH_3), 4.08 (ddd, 1H, $J_{5,6}$ = 1.0 Hz, *H*-6), 4.15 (td, 1H, $J_{3,4}$ = 4.0 Hz, *H*-3), 4.55 (dd, 1H, $J_{4,5}$ = 6.0 Hz, *H*-5), 4.72 (dd, 1H, *H*-4), 5.01 (dd, 1H, N*H*), 7.58-7.63 (m, 3H, *H*-Ar), 7.88-7.93 (m, 2H, *H*-Ar). ^{13}C NMR (62.9 MHz, $CDCl_3$): δ = 25.1 (*C*H_3), 26.3 (*C*H_3), 34.0 (*C*-2), 42.7 (*C*-7), 52.0 (O*C*H_3), 76.5 (*C*-3), 81.1, 81.9, 83.1 (*C*-4, *C*-5, *C*-6), 100.3 (*C*-Ar-I), 113.3 (*C*$(CH_3)_2$), 128.6 (2 *C*-Ar), 138.6 (2 *C*-Ar), 139.4 (*C*-Ar), 171.5 (*C*=O). IR (film) υ: 3279, 2985, 2938, 1735, 1572. ESI-HRMS: m/z calcd for $C_{17}H_{22}NO_7SINa$ $[M+Na]^+$ 534.0054; found 534.0056.

3,6-Anhydro-2,7-deoxy-4,5-*O*-(1-methylethylidene)-7-[[4-(1-trimethylsilylacetylenyl)benzo]sulfonylamino]-D-*altro*-heptonic acid 65

To a stirred solution of **64** (137 mg, 0.25 mmol, 1 eq.), $PdCl_2(PPh_3)_2$ (18 mg, 0.025 mmol, 0.1 eq.) and CuI (3 mg, 0.015 mmol, 0.06 eq.) in Et_3N (1 mL) was added ethynyltrimethylsilane (42 µL, 0.3 mmol, 1.2 eq.). The mixture was stirred at 80 °C until completion of the reaction. The solvent was removed under reduced pressure and the crude was purified by flash chromatography (silica gel, cyH/EA) to provide **65** (96 mg, 80%).Colorless oil. *Rf;* 0.32 (cH/EA: 7/3). $[\alpha]_D$= +20.2° (c = 0.2, $CHCl_3$). ^{1}H NMR (250 MHz, $CDCl_3$): δ = 0.29 (s, 9H, C(CH_3)$_3$), 1.32 (s, 3H, C(CH_3)$_2$), 1.47 (s, 3H, C(CH_3)$_2$), 2.67 (dd, 1H, J_{gem} = 17.0, $J_{2,3}$ = 7.0 Hz, *H*-2), 2.76 (dd, 1H, $J_{2',3}$ = 7.0 Hz, *H*-2'), 2.89 (ddd, 1H, J_{gem} = 12.0, $J_{6,7}$ = 9.0, $J_{7,NH}$ = 3.5 Hz, *H*-7), 3.08 (ddd, 1H, $J_{6,7'}$ = 5.0, $J_{7',NH}$ = 8.0 Hz, *H*-7'), 3.74 (s, 3H, OCH_3), 4.07 (ddd, 1H, $J_{5,6}$ = 1.0 Hz, *H*-6), 4.13 (td, 1H, $J_{3,4}$ = 4.0 Hz, *H*-3), 4.54 (dd, 1H, $J_{4,5}$ = 6.0 Hz, *H*-5), 4.72 (dd, 1H, *H*-4), 4.87 (dd, 1H, N*H*), 7.61 (d app, 2H, *J* = 8.5 Hz, *H*-Ar), 7.82 (d app, 2H, *H*-Ar). ^{13}C NMR (62.9 MHz, $CDCl_3$): δ = 0.0 (C(*C*H_3)$_3$), 25.2 (*C*H_3), 26.4 (*C*H_3), 34.1 (*C*-2), 42.8 (*C*-7), 52.1 (O*C*H_3), 76.6 (*C*-3), 81.2, 82.0, 83.2 (*C*-4, *C*-5, *C*-6), 98.9, 103.3 (*C*≡*C*), 113.4 (*C*$(CH_3)_2$), 127.1 (*C*-Ar), 127.2 (*C*-Ar), 128.3 (*C*-Ar), 132.7 (*C*-Ar), 132.8 (*C*-Ar), 139.1 (*C*-Ar), 171.5 (*C*=O). IR (film) υ: 3279, 2995, 2957, 2900, 2156, 1735, 1588. ESI-HRMS: m/z calcd for $C_{22}H_{31}NO_7SSiNa$ $[M+Na]^+$ 504.1483; found 504.1479.

3,6-Anhydro-2,7-deoxy-4,5-*O*-(1-methylethylidene)-7-[[(4-acetylenyl)benzo]sulfonylamino]-D-*altro*-heptonic acid 66

Prepared starting from **65** (80 mg, 0.17 mmol) following procedure described for **21**. Yield: 82% (55 mg). White foam. *Rf;* 0.48 (EA). $[\alpha]_D$= +27.5° (c = 0.1, $CHCl_3$). ^{1}H NMR (250 MHz, $CDCl_3$): δ = 1.30 (s, 3H, C(CH_3)$_2$), 1.46 (s, 3H, C(CH_3)$_2$), 2.70 (dd, 1H, J_{gem} = 17.0, $J_{2,3}$ = 7.0 Hz, *H*-2), 2.78 (dd, 1H, $J_{2',3}$ = 6.0 Hz, *H*-2'), 2.90 (ddd, 1H, J_{gem} = 13.0, $J_{6,7}$ = 8.5, $J_{7,NH}$ = 4.0 Hz, *H*-7), 3.08 (ddd, 1H, $J_{6,7'}$ = 5.0, $J_{7',NH}$ = 8.0 Hz, *H*-7'), 3.26 (s, 1H, *H*-C≡C), 4.09 (m, 1H, *H*-6), 4.15 (m, 1H, *H*-3), 4.55 (dd, 1H, $J_{4,5}$ = 6.0, $J_{5,6}$ = 1.0 Hz, *H*-5), 4.71 (dd, 1H, $J_{3,4}$ = 4.0 Hz, *H*-4), 5.20 (dd, 1H, N*H*), 7.59-7.63 (m, 2H, *H*-Ar), 7.79-7.83 (m, 2H, *H*-Ar). ^{13}C NMR (62.9 MHz, $CDCl_3$): δ = 24.9 (*C*H_3), 26.1 (*C*H_3), 34.0 (*C*-2), 42.6 (*C*-7), 76.3 (*C*-3), 80.8, 80.9, 81.9, 83.0 (*C*-4, *C*-5, *C*-6, *C*≡*C*), 113.3 (*C*$(CH_3)_2$), 127.0 (3 *C*-Ar), 132.8 (2 *C*-Ar), 139.5 (*C*-Ar), 175.4 (*C*=O). IR (film) υ: 3270, 2990, 2928, 1711. ESI-HRMS: m/z calcd for $C_{18}H_{21}NO_7SNa$ $[M+Na]^+$ 418.0931; found 418.0941.

***N*$^\alpha$-[2-*C*-[2,3-*O*-(1-methylethylidene)-5-[((4-acetylenyl)benzo)sulfonylamino]-α-D-ribofuranosyl]carbonylmethyl)]]-L-arginine methyl ester 67**

Prepared starting from **66** (45 mg, 0.11 mmol) following procedure described for **60**. Yield: 67% (42 mg). White solid. *Rf;* 0.12 (H_2O/MeOH: 1/1). $[\alpha]_D$= +10.6° (c = 0.3, H_2O). ^{1}H NMR (400 MHz, D_2O): δ = 1.23 (s, 3H, C(CH_3)$_2$), 1.39 (s, 3H, C(CH_3)$_2$), 1.46-1.54 (m, 2H, CH_2 *arg*), 1.62 (m, 1H, CH_2 *arg*), 1.77 (m, 1H, CH_2 *arg*), 2.49 (dd, 1H, J_{gem} = 15.0, $J_{2,3}$ = 7.0 Hz, *H*-2), 2.58 (dd, 1H, $J_{2',3}$ = 7.0 Hz, *H*-2'), 2.86 (dd, 1H, J_{gem} = 13.5, $J_{6,7}$ = 7.0 Hz, *H*-7), 2.91 (dd, 1H, $J_{6,7'}$ = 7.0 Hz, *H*-7'), 3.07 (t, 2H, *J* = 7.0 Hz, CH_2 *arg*), 3.95 (t, 1H, *H*-6), 4.07 (td, 1H, $J_{3,4}$ = 4.0

Hz, *H*-3), 4.11 (dd, 1H, $J_{H\text{-}\alpha,A1}$ = 8.0, $J_{H\text{-}\alpha,A1'}$ = 4.5 Hz, *H*-α), 4.57 (d, 1H, $J_{4,5}$ = 6.0 Hz, *H*-5), 4.67 (dd, 1H, *H*-4), 7.62 (d app., 2H, *J* = 8.0 Hz, *H*-Ar), 7.72 (d app., 2H, *H*-Ar). ^{13}C NMR (100 MHz, D_2O): δ = 23.5 (*C*H_3), 24.4 (*C*H_2 *arg*), 24.9 (*C*H_3), 28.8 (*C*H_2 *arg*), 35.3 (*C*-2), 40.6 (*C*H_2 *arg*), 42.7 (*C*-7), 54.5 (*C*-α), 76.7 (*C*-3), 80.6 (*C*-4), 81.9 (*C*≡C), 82.6, 82.7 (*C*-5, *C*-6), 112.9 (*C*(CH_3)$_2$), 125.8 (*C*-Ar), 126.6 (2 *C*-Ar), 132.8 (2 *C*-Ar), 140.4 (*C*-Ar), 156.6 (*C*=N), 171.7 (*C*=O), 178.5 (*C*=O). IR (pellets) υ: 3350, 3289, 3184, 2990, 2938, 2872, 1648, 1593. ESI-HRMS: m/z calcd for $C_{24}H_{33}N_5O_8SNa$ $[M+Na]^+$ 574.1942; found 574.1960.

Compound 69

To a solution of alcyne **67** (33 mg, 0.04 mmol, 1 eq.) and azido sugar **68** (15 mg, 0.04 mmol, 1 eq.) in a water/*t*BuOH mixture (0.5 mL/0.5 mL) were added sodium ascorbate (2.4 mg, 0.008 mmol, 0.2 eq.) and $Cu(OAc)_2$ (1.2 mg, 0.004 mmol, 0.1 eq.). The solution turned progressively pale green and the mixture was stirred at room temperature until completion of the reaction (the blue color reappeared). Chelex® resin (100 mg) was then added to the solution and the suspension was stirred until the solution became colorless. The resin was filtered off and the solvent were removed under reduced pressure. The crude was purified by column chromatography (C-18 silica gel, H_2O/MeOH) and lyophilized to provide **69** (37 mg, 77%).White solid. *Rf;* 0.29 (H_2O/MeOH: 1/1). HPLC (70/30): t_R = 4.180 min. $[\alpha]_D$= -6.7° (c = 0.1, H_2O). ^{1}H NMR (400 MHz, D_2O): δ = 1.31 (s, 3H, C(CH_3)$_2$), 1.46 (s, 3H, C(CH_3)$_2$), 1.53-1.61 (m, 2H, CH_2 *arg*), 1.69 (m, 1H, CH_2 *arg*), 1.84 (m, 1H, CH_2 *arg*), 2.59 (dd, 1H, J_{gem} = 15.0, $J_{2,3}$ = 8.0 Hz, *H*-2), 2.66 (dd, 1H, $J_{2',3}$ = 6.5 Hz, *H*-2'), 3.04 (dd, 1H, J_{gem} = 13.5, $J_{6,7}$ = 8.5 Hz, *H*-7), 3.08-3.17 (m, 3H, CH_2 *arg*, *H*-7'), 3.26 (m, 1H, *H*-11), 3.42- 3.59 (m, 3H, *H*-12, *H*-13, *H*-14), 4.08 (dd, 1H, $J_{6,7}$ = 8.5, $J_{6,7'}$ = 6.5 Hz, *H*-6), 4.14-4.25 (m, 3H, *H*-3, *H*-α, *H*-9), 4.33 (m, 1H, *H*-9'), 4.47 (d, 1H, $J_{10,11}$ = 8.0 Hz, *H*-10), 4.61 (dd, 2H, $J_{H,F}$ = 47.5, $J_{14,15}$ = 2.0 Hz, *H*-15), 4.68 (d, 1H, $J_{4,5}$ = 6.0 Hz, *H*-5), 4.73-4.77 (m, 3H, *H*-4, 2 *H*-8), 7.94 (d app., 2H, *J* = 8.0 Hz, *H*-Ar), 7.99 (d app., 2H, *H*-Ar), 8.49 (*H*-triazole). ^{13}C NMR (62.9 MHz, D_2O): δ = 23.9 (*C*H_3), 24.6 (*C*H_2 *arg*), 25.3 (*C*H_3), 29.1 (*C*H_2 *arg*), 35.6 (*C*-2), 40.9 (*C*H_2 *arg*), 42.2 (*C*-7), 50.9 (*C*-8), 54.8 (*C*-α), 68.5 (d, $J_{C,F}$ = 7.0 Hz, *C*-13), 68.7 (*C*-9), 73.1 (*C*-11), 74.5 (d, $J_{C,F}$ = 18.0 Hz, *C*-14), 75.7 (*C*-12), 77.0 (*C*-3), 81.0, 82.4, 82.7 (*C*-4, *C*-5, *C*-6), 82.1 (d, $J_{C,F}$ = 168.0 Hz, *C*-15), 102.9 (*C*-10), 113.3 (*C*(CH_3)$_2$), 124.4 (*C*-Ar), 126.7 (2 *C*-Ar), 127.8 (2 *C*-Ar), 134.7 (*C*-triazole), 138.5 (*C*-Ar), 146.0 (*C*-triazole), 156.9 (*C*=N), 172.0 (*C*=O), 178.7 (*C*=O) ^{19}F NMR (235.2 MHz, D_2O): δ = -235.16 (td, $J_{H,F}$ = 47.0, $J_{H,F}$ = 27.0 Hz). IR (ATR) υ: 3346, 3236, 1655, 1505, 1150. ESI-HRMS: m/z calcd for $C_{32}H_{47}FN_8O_{13}SNa$ $[M+Na]^+$ 825.2860; found 825.2845.

Molecular modeling

The structures of the investigated compounds were prepared according to the CORINA[1] (for the 3D conformers) and MOPAC[2] softwares (for the atomic charges). The chemicals were firstly docked using the GOLD software.[3] The protein target was the 3D structure of the b1 domain of NRP-1 as solved by X-ray when bound with the small molecule EG00229[4] (pdb code 3I97). Previous to the docking process, all hydrogen atoms were added to the PDB protein chain A considered at pH 7. In the GOLD settings, the protein binding site was defined according to the position of the EG00229 ligand within the complex providing a list of interacting amino acid residues with an extra range of 10 Å from each ligand atom. For each docking run, 50 different ligand starting poses were used and optimized according to the Goldscore docking function. Molecular dynamics simulations were performed using the NAMD software.[5] All protein/ligand complexes resulting from the best GOLD docking poses were checked for their stability using short 10 ns molecular dynamics. For this purpose, each protein 3D model was first solvated with an 80 Å3 box of TIP3P explicit water molecules. Next, ions were added for ensuring the electrostatic neutrality of the whole protein+solvent systems. The initial states for dynamics were generated from the GOLD models after 64,000 steps of conjugate gradients minimization followed by an equilibration stage of 1 ns. The simulations were carried out in the isobaric-isothermal ensemble, maintaining the pressure and the temperature at 1 atm and 300 K respectively by using Langevin dynamics and the Langevin piston approaches. The equations of motion were integrated with a 1fs time step. Long-range interactions were treated using the particle-mesh Ewald approach with an 11 Å cut-off (switching distance 9 Å) for the real space calculation. The calculation of forces and motion equations was repeated to generate the trajectories corresponding to the requested simulation times. A conformation of the whole molecular system was recorded every 10ps, generating for each 10 ns runs a conformational sample of 1,000 frames which were analyzed using the VMD graphic software.[6]

Protein production, purification and biocrystallogrphy

Recombinant pET-15b (Novagen) vector producing the human NRP-1-b1 domain protein were previously constructed using the NdeI and BamHI restriction sites. The recombinant *N*-terminal His-fusion protein containing a bovine thrombin protease site were produced in *E*. coli cells strain BL21 (DE3) Rosetta 2 (Novagen). Growth was performed in 2 L of Luria Broth medium under agitation at 37°C and induced to an OD of 0.8 by addition of 0.2 mM Isopropyl-β-D-Thiogalactopyranoside (Euromedex) and shifted at 20°C under the night. Cell harvesting was carried out by centrifugation (4 000 g, 30 min, 4°C). For purification, the frozen pellet was resuspended in 30 ml of lysis buffer (TrisHCl 50 mM pH 8, NaCl 300 mM, Imidazole 20 mM, β-Mercaptoethanol 5 mM) for sonication. After a centrifugation of 30 min at 4°C, 37 000 g, the supernatant was injected on a HiTrap TALON crude 5 ml column (GE Healthcare) and eluted by an imidazole gradient. The eluate was injected on a Hiload 16/60 Superdex 75 prep grade column (GE Healthcare) in GF buffer (TrisHCl 20 mM pH 8, NaCl 50 mM, Tris 2-CarboxylEthyl Phosphine 0.5 mM) and the adequate fractions were pooled and concentrated using an Amicon Ultra-15 (Merck Millipore, 10 kDa cut-off) to a concentration of about 40 mg/ml.

A crystallization screening ofNRP-1-b1 fragment including a 6-His tag at the *N*-terminus was carried out in presence of compounds **27**, **40**, **50** and **56**. The protein was diluted to a concentration of 7mg/ml and 10mM ligand prior to crystallization with the hanging drop method at 20°C. Crystals were obtained by mixing 2µl of protein solution with 1.5 µl of precipitating solution from the well: 25% PEG 550 mme, 5% PEG 20 000, 60mM $MgCl_2$, Na-Bicine 100mM pH 8.5. Diffraction data were collected at BM30A beamline at ESRF (Grenoble, France) at a wavelength of 0.97967 Å using a ADSC Quantum 315r detector. The diffraction intensities were indexed, integrated, scaled and merged with XDS and XSCALE.[7] The structure, including modelled protein atoms, was refined with PHENIX[8] starting from the coordinates of the 1KEX PDB entry.[9] The crystal structure has been deposited at the Protein Data Bank[10] with code 5C7G.

Biological Assay

General procedure for receptor binding assays: NRP-1 and KDR were obtained from R&D Systems (Lille, France), as recombinant chimeric proteins. The binding of new derivatives to recombinant NRP-1 protein was determined using a competition assay initially described by Tirand *et al. (see details in SI).*[11]The surface of microplates (Dutscher) was coated with either NRP-1 (2 mg/mL) or KDR (2 mg/mL) in PBS, overnight at room temperature. The plates were blocked with PBS containing 0.5% bovine serum albumin (blocking buffer) during 1 h at 37°C, to prevent non-specific interactions. Binding of compounds to NRP-1 was assessed using 5 ng/mL of biotinylated VEGF-A_{165} (R&D Systems) in blocking buffer containing 2 mg/mL heparin. Biotinylated VEGF-A_{165} was added to the coated wells, in competition, or not, with an excess of compounds or non labelled VEGF-A_{165} (R&D Systems), as a positive control. After a 2-h incubation at room temperature, the plates were washed and the amount of bound biotinylated VEGF-A_{165} stained with streptavidin horseradish peroxidase conjugate (R&D Systems). After 20 min at room temperature, reaction was stopped by the addition of Stop Solution (R&D Systems). Optical densities were measured at 450 nm. Results were expressed as relative absorbance to wells containing only blocking buffer. Three wells per condition were used.

General procedure for HUVECs culture and treatments: Briefly, HUVECs were collected from umbilical cords as previously described by Jaffe *et al* (see details in SI).[12] HUVECs were cultured and used until passage 3 in EndoGro medium (Merck-Millipore, France) supplemented with 10% calf serum, 2 mM l-glutamine (GibcoBRL), 100 U/ml penicillin (GibcoBRL, France), 100 µg/ml streptomycin (GibcoBRL, France), 2.5 µg/ml amphothericin B (GibcoBRL, France). For all experiments, tuftsin (Bachem, Switzerland), (TKPR) the natural ligand of NRP-1, was used as reference control. For angiogenesis and migration assays, bevacizumab (Roche, France) was used as positive control at the concentration of 200 µg/mL.

General procedure for NRP1 signaling pathways: For analysis of pAKT and pERK1/2 expression, western blotting was realized as previously described (see details in SI).[13] Briefly, protein aliquots (40 µg) were denatured in the Laemmli buffer, containing ß-mercaptoethanol and before to be resolved in SDS-polyacrylamide gel electrophoresis. The separated proteins were transferred onto PVDF membranes (Amersham Biosciences, Orsay, France). After blocking the PVDF membrane with Tris base-buffered saline prepared with 0.1% (v/v) Tween-20 containing 5% (w/v) bovine serum albumin, the following primary antibodies against human AKT (#9272, Cell Signaling, 1:1000 dilution), phosphorylated AKT at ser473 (pAKT) (#9271, Cell Signaling, 1:1000 dilution), ERK1/2 (#9102, Cell Signaling, 1:1000 dilution), phosphorylated ERK1/2 at Thr202/Tyr204 (pERK1/2) (#9106, Cell Signaling, 1:1000 dilution) were incubated overnight at 4˚C. Subsequently, immuno-reactive proteins were visualized using the enhanced chemiluminescence procedure (Pierce, USA). Quantification of relative band densities was performed using densitometer (LAS Imager FujiFilm) and AKT and ERK were used as control.

General procedure for cell viability assays: Roche's WST-1 cell proliferation reagent is a simple, colorimetric assay designed to measure the relative proliferation rates of cells in culture (see details in SI). The assay principle is based on the conversion of the tetrazolium salt WST-1 into a colored dye by mitochondrial dehydrogenase enzymes. The soluble salt is released into the media. Within a given time period, the reaction produces a color change which is directly proportional to the amount of mitochondrial dehydrogenase in a given culture. As a result, the assay actually measures the net metabolic activity of cells. To perform the assay, the ready-to-

use WST-1 reagent is simply added directly into the media of cells cultured in 96 well plates. The cultures are then given 30 minutes to reduce the reagent into the dye form. At the set time, 0.5 µmol/L WST-1 were added into each well. WST-1 reagent was used according to manufacturer instructions. The absorbance of the resulting solution was measured at 450 nm using wells without cells as blank (Multiskan Ascent microplate photometer, Thermo Scientific, France).

Angiogenesis assay: HUVECs were plated (90,000 cells/cm2) onto 24-well plate ibidi precoated with Matrigel™ Basement Membrane Matrix (BD Biosciences, France, see details in SI). After 1 h, compounds were added on HUVECs which were cultured during overnight before being fixed with 4% paraformaldehyde. Photomicrographs of whole culture surface were taken (Nikon AZ100, Digital Sight DS-Qi1Mc camera, Nikon, France). The number of junctions and segments as well as the network length was quantified with Angiogenesis Analyzer for ImageJ.[14]

Invasion assay: The invasion ability of cells was analyzed using culture permeable support (8 µm pore size, Falcon) coated with 100 µL Matrigel™ (BD Biosciences). HUVECs were pretreated with the different compounds during 24h and plated at a density of 4.105 cells in 500 µL serum free medium, inoculated in the upper chamber; while 700 µL of medium containing 10% FBS was used as the chemoattractant was placed in the lower chamber. After incubating the cells for 24h à 37°C, the non-invading cells remaining on the upper side of the filter were gently removed with cotton swab. The invading cells on the lower chamber were fixed with 4% paraformaldehyde (PFA) for 10 min and stained with crystal violet for 30 min. Crystal violet was solubilized with 4% acetic acid and absorbance at 540 nm was measured. All results were given as mean ± standard error of the mean (SEM) was used. Unpaired t test was employed to determine the statistical significance with a limit set to $p<0.05$ using GraphPad Prism 5 (GraphPad Software, US) versus non treated cells.

Table S1. Crystallographic summary

Diffraction data	
Space group	P $4_1 2_1 2$
Unit cell *a*, *c* (Å)	62.280 85.960*
Wavelength (Å)	0.97967
Resolution (Å)	1.45
Unique reflections	30 507
Average redundacy	12.2
R_{meas}(%)	5.1
Completeness (%)	99.5
Refinement	
R-factor (%)	14.1
R-free (%)	17.2
# Protein atoms	2431
# water atoms	294
Wilson *B*-factor (Å^2)	26.4
RMSD from target	
Bond lengths (Å)	0008
Bond angles (deg)	1.26

* The *c* unit cell axis is significantly shorter (85.96 vs. 88.375 Å) compared to the published free protein fragment structure[9]

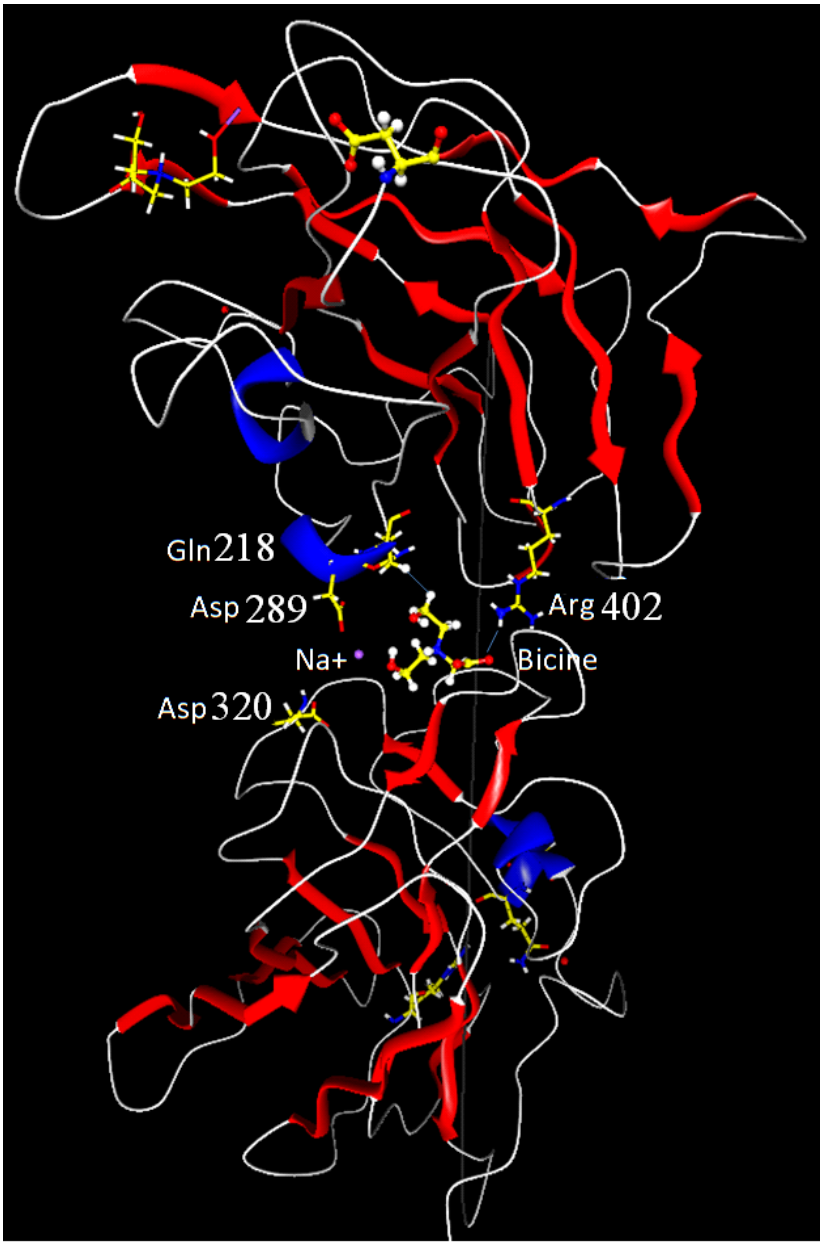


Figure S1.

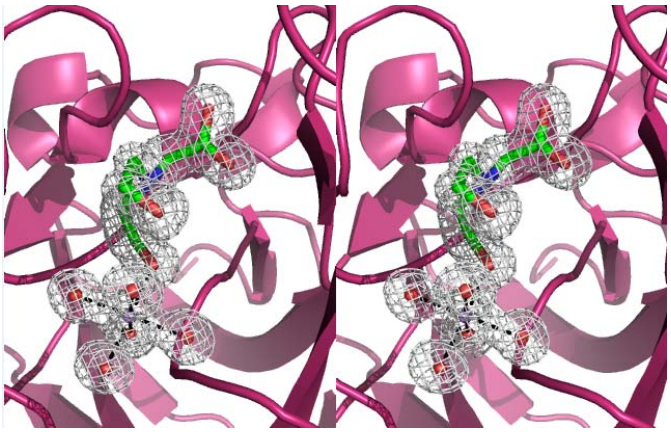
Ribbon view of the NRP-1 b1 dimer in the tetragonal crystal, where bicine interacts with residues of a symmetry related protein molecule. Symmetry code: -*X*+½, *Y*-½, -*Z*+¼. Some residues interacting with bicine and the Na^+ ion are shown.

The bicine molecule forms short H...H van der Waals contacts (d = 2.23 Å) with the symmetry related Gln218 side chain. The bicine carboxylate group is also making a salt bridge with the symmetry related Arg402.

The binding of bulky ligands in the cleft is not compatible with the formation of the present tetragonal crystals. The binding cleft is located too close to a symmetry related protein. The b1 domain of human NRP-1 bound with bulky molecule EG00229 crystallized actually in a different space group with two molecules in the asymmetric unit.[4]

The bicine molecule was used as pH buffer at a concentration of 100mM in the conditions yielding crystals. The initial structure of the free protein[9] which was crystallized at pH 6 using MES buffer has only water molecules in the binding site.

a)



b)

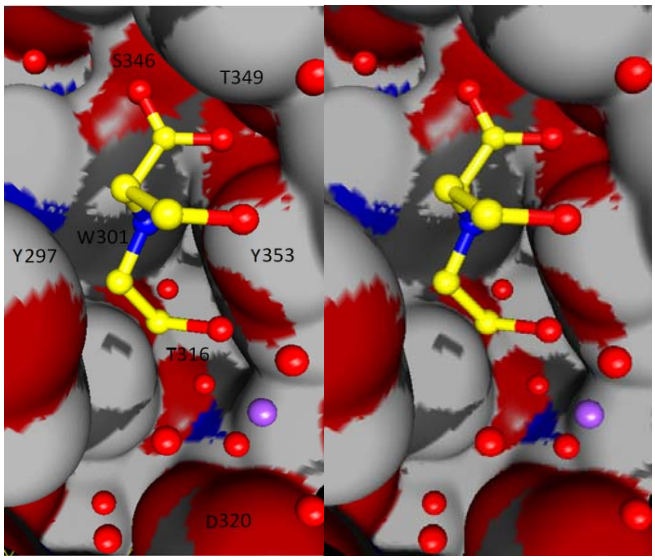


Figure S2. a) Stereo view (wall eyes) of the electron density showing the bicine molecule in the binding site. The map shown is σ_A-weighted 2mF_o-DF_c map contoured at 1.2σ.
b) Stereoview (wall eyes) of the bicine molecule within the binding site surface. The protein-only surface is shown at an electron density level of 0.01 e/Å^3 and colored according to atom types (oxygen: red, nitrogen: blue, carbon: grey, hydrogen: white). The water molecules are shown as red spheres and the Na^+ ion as a purple sphere. The image was created with MoProViewer.[15] The Na^+ ion is also stabilized in this position distant from Asp320 due to a second electrostatic interaction with the symmetry related carboxylate of Asp289 (d = 4.1 Å)

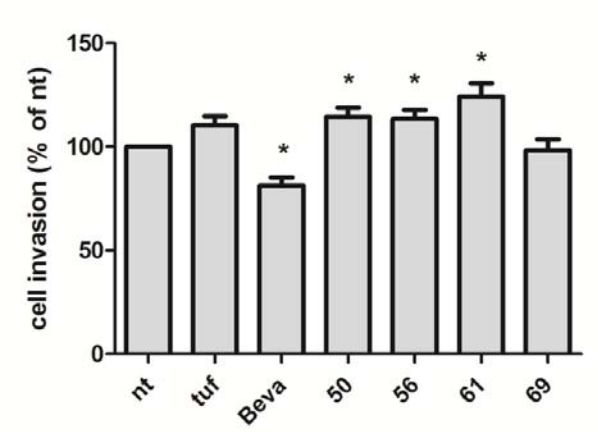


Figure S3: Effect of the different compounds (c = 50 µM) on HUVECs ability to migrate through a basement membrane matrix (invasion assay). The results are presented as mean ± standard error of the mean of the percentage of inviding cells with non treated cells used as reference, n=6 $p<0.05$ was considered to be statistically significant. tuf, tuftsin; beva, bevacizumab.

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